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# Bioethanol production from cellulosic substrates: Engineered bacteria and process integration challenges

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Cellulosic biomass from agricultural and forestry residues, waste paper and industrial wastes could be used as an ideal and inexpensive source of sugar for sustainable fermentation into transportation fuel. As such, ethanol-producing microorganisms, mainly *Zymomonas mobilis* and *Saccharomyces cerevisiae* are potential candidates for ethanol production. However, the substrates are not cost effective, as the organisms are not able to hydrolyze complex sugars such as lignocellulose. Since last two decades, several microorganisms are manipulated for production of ethanol. Gram-negative bacteria such as *Escherichia coli*, *Klebsiella oxytoca*, *Z. mobilis,* Gram-positive bacteria such as *Clostridium cellulolyticum*, *Lactobacillus casei* and several yeast strains have been engineered for bioethanol production from cellulosic substrates. These engineered organisms are able to produce ethanol from a wide spectrum of sugars. This review is focused on the strategies and development of processes for ethanol production by such organisms from lignocellulosic substrates.

**Keywords**: Bioethanol, Cellulosic biomass, Ethanol producing bacteria, Metabolic engineering
**IPC Code**: C10L1/02

## Introduction

Countries dependence on imported oil, environmental issues, and employment in rural areas are reasons for replacement of fossil fuels by bioethanol. Out of world ethanol production[1] of approx 31.2 billion liters, 7 percent corresponds to synthetic alcohol derived from gas or coal. Bioethanol accounts for approx two-thirds of the total production. Brazil is the largest producer of bioethanol (13.5 billion l); USA attained 6.4 billion liters. Brazilian bioethanol is totally channeled to the fuel sector, whereas in USA approx 3.9 billion liters is used for the domestic fuel mix. In European Union (EU), of total ethanol produced (> 2 billion l), major share belongs to: France, 30 (120 million l); UK, 18; Germany, 17; and Italy, 9%. Of total ethanol produced in EU, only 5 percent is used as fuel. Synthetic alcohol predominates in Germany and UK. Sweden used 12 million liters of bioethanol, which corresponds to about 0.22 percent of the 5.5 billion liters of gasoline consumed[2]. It is expected that the demand[3] for ethanol for transportation will increase dramatically until 2010.

Ethanol producing bacteria (EPB) have attracted much attention because of their growth rate higher than that of *Saccharomyces cerevisiae,* conventionally used organism for commercial production of bioethanol. EPB are expected to make industrial ethanol production more economical. EPB *Zymomonas mobilis* has been used in tropical areas to make alcoholic beverages from plant sap[4], but its narrow spectrum of fermentable carbohydrates has hampered its industrial exploitation[5]. To overcome this limitation, attempts have been made to expand the substrate range of *Z. mobilis*[6-8]. A number of groups have taken on the challenge of developing recombinant organisms, including *S. cerevisiae*, *Z. mobilis*, *Escherichia coli*, *Klebsiella oxytoca* and *Erwinia herbicola*[9-11] but the production of ethanol from biomass materials by genetically engineered strains has not yet reached a level sufficient for commercial application. For this reason, *Z. mobilis* was genetically engineered to produce ethanol directly from cellulosic materials[12].

Pretreating biomass completely or partially hydrolyzes the hemicellulose, removes lignin and de-crystallizes cellulose, thereby allowing cellulase enzymes access to cellulose fibers. Pretreated cellulose can be enzymatically hydrolyzed either prior to fermentation in sequential saccharification and fermentation or by adding the cellulase and inoculum together as in simultaneous saccharification and

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Table 1—Important traits for ethanol fermentation process

| Parameters | Desired level |
|---|---|
| Ethanol yield, g/g | >90% of theoretical yeild |
| Ethanol tolerance, g/l | >40g/l |
| Ethanol productivity, g/l/h | >1g/l/h |
| Simple growth medium | Inexpensive medium formulation |
| Able to grow in concentrated substrates | Resistance to inhibitors |
| Culture growth conditions retard contaminants | Acidic pH or higher temperatures |

fermentation (SSF), which gives higher ethanol yield. This is because end product inhibition from cellobiose and glucose formed during enzymatic hydrolysis is relieved by fermentation. SSF with yeast and cellulases are typically carried out at pH 4.5 and 37°C.

High ethanol yield, low byproducts, and metabolize a wide range of major sugars are the desired characteristics (Table 1) for industrial ethanol production[13,14]. The engineered ethanologenic bacteria that currently show the most promise for industrial exploitation are *E. coli*, *K. oxytoca*, *Z. mobilis*, *Clostridium cellulolyticum* and *Lactobacillus casei*. However, the performance of these strains is not yet reached to industrial scale.

## Cellulosic Substrates for Bioethanol Production

In contrast to sugar-containing crops, utilization of lignocellulose as a substrate for ethanol production has a barrier in its complex structure, which resists degradation. Lignocellulose[14] is composed of (dry weight): cellulose, 45; hemicellulose 30; and lignin 25%. Cellulose, the most abundant polymer on earth, is composed of fibrous bundles of crystalline cellulose encased in a polymeric matrix of hemicellulose and lignin. The basic repeating unit is the disaccharide cellobiose. The secondary and tertiary conformation of cellulose, as well as its close association with lignin, hemicellulose, starch, protein and mineral elements, makes its rigidity. Cellulose can be hydrolyzed enzymatically or with acid. Hemicellulose is a highly branched heteropolymer containing hexoses (D-galactose, L-galactose, D-mannose, L-rhamnose, L-fructose), pentoses (D-xylose, L-arabinose), and uronic acids (D-glucuronicacid). Its composition depends on the source of raw material[14]. Lignin, the most abundant aromatic polymer in nature, is a macromolecule of phenolic character, being the dehydration product of three monomeric alcohols (lignols), *trans-p*-coumaryl alcohol, *trans-p*-coniferyl alcohol, and *trans-p*-sinapyl alcohol, derived from *p*-cinnamic acid[15].

## Engineering *E. coli* for Ethanol Production from Cellulosic Substrates

The construction of *E. coli* strains to selectively produce ethanol was one of the first successful applications of metabolic engineering[16]. *E. coli*, as a biocatalyst for ethanol production, has ability to ferment a wide spectrum of sugars, no requirements for complex growth factors, and prior industrial use (for production of recombinant protein). *E. coli* strains were evaluated as hosts for ethanol production[17]. The major parameter used for screening in xylose fermentation included ethanol tolerance, plasmid stability and ethanol yields. *E. coli* ATCC11303 (pLOI297) is reported to be the most promising strain for xylose fermentations under a wide-variety of culture conditions[18]. Under the optimum fermentation conditions (temp <42°C, pH>6.0, xylose 80 g/l), xylose-fermenting cultures showed maximum ethanol tolerance (53-56 g/l) and an average productivity (0.72 g/l/h).

Plasmids carrying alcohol dehydrogenease *(adh)* and pyruvate decarboxylase *(pdc)* genes are unstable in the absence of antibiotics, these strains are unstable for use in industrial fermentations. Therefore, genes encoding these key enzymes were integrated into the chromosome of strain ATCC11303[19]. The *pet* operon was inserted into the pyruvate formate lyase (*pfl*) gene, in an attempt to eliminate an enzyme competing for pyruvate. However, resulting construct yielded lower amounts of ethanol than the plasmid-bearing strains, attributed to reduced gene dosage. Chloramphenicol (Cm) resistance (40–600 mg/l) screening strategy was developed for selecting mutants with increased *adh* and *pdc* expression. The hyper-resistant mutants were again screened on acetaldehyde selection plates and thus hyper-ethanol producing strains were successfully recovered. Disrupting terminal gene in the succinate pathway, fumarate reductase (*frd*), eliminated succinate production and further increased ethanol yield. The resultant strain KO11 grows faster on xylose-containing medium than its parent strain ATCC11303. Comparison of global gene expression by microarray technology, demonstrated that KO11 over-expresses genes of xylose metabolism[20]. The performance of strain KO11 has been evaluated for fermentation of hemicellulose hydrolysates[21,22] from *Pinus* wood, sugarcane bagasse and corn stover. Dumsday *et al*[23] carried out the most comprehensive culture studies with KO11 grown in continuous cultures. High ethanol

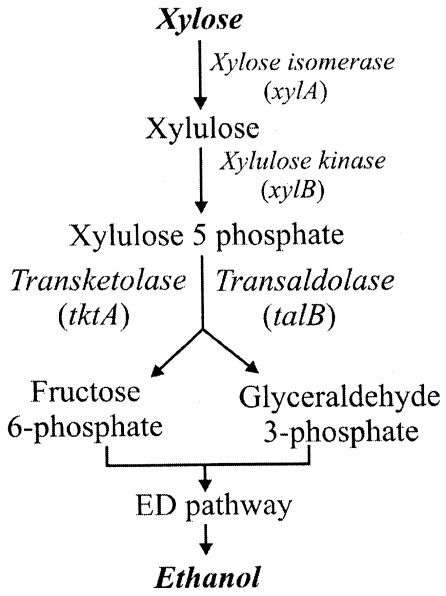


Fig. 1—Xylose metabolism of recombinant *Zymomonas mobilis* The four *E. coli* genes of xylose metabolism were introduced to utilize xylose.

yields were maintained in glucose-fed cultures, but not in xylose or glucose/xylose mixtures. When xylose alone was used as the carbon source (xylose, 20g/l; dilution rate, 0.06/h), ethanol yields declined after 5 days and cells screened from the continuous culture began to lose Cm hyper-resistance (600 mg/l) after 30 days. However, the instability is a hindrance only for utilizing KO11 in continuous fermentation processes. The lower ethanol yield of KO11 was reported due to genetic instability of the strain[24,25].

FBR strains were constructed using *pfl* and *ldh* mutant strains that had block in their ability of the strains to reduce pyruvate and recycle the NADH, $H^+$ generated from glycolysis. Transforming the strains with plasmid pLOI297, carrying *pet* operon, restored fermentative growth. The transformed strains selectively produced ethanol from arabinose, glucose or xylose. Furthermore, pLOI297 is positively maintained in anaerobic culture[26,27] because cells that lose the plasmid cannot grow. These strains have been used to ferment hydrolysates prepared from corn hulls and germ meal[28,29]. Best strain, FBR5, produced ethanol (0.46-0.51g/g), and fermentation was complete within 36-60h[29]. Variants of these strains[30] have been constructed-mutants that are not repressed by glucose because they all carry a mutation in their phosphoenolpyruvate-glucose phosphotransferase system (*ptsG*-). These strains have the ability to utilize arabinose, glucose and xylose simultaneously. However, *ptsG*- also disables active glucose transport in *E. coli*. As a result, mutants grow slower on glucose and are more sensitive than FBR5 to inhibitors present in corn hull hydrolysates.

Long-term adaptation of KO11 in medium supplemented with ethanol increased ethanol tolerance[31]. Serial adaptation of strain over 3 months in LB medium, supplemented with glucose (50 g/l) and xylose (140 g/l), elevated ethanol (35–50g/l) and reduced the time from 120h to 96h[31]. LY01 tolerated hydrolysate-associated inhibitors, including aldehydes[32], alcohols and organic acids[33], better than KO11. Cultures of LY01 were able to tolerate up to 25g/l acetic acid and 3.5g/l each of HMF (hydroxymethylfurfural) and furfural.

Currently, the focus is on the formulation of inexpensive culture medium and improving inhibitor tolerance level of KO11. Higher ethanol yields was obtained from the medium containing corn steep liquor (15g/l) and yeast autolysate (4g/l) than from LB medium (0.43 vs. 0.48g/g), albeit with a decline in productivity (from 0.80 to 0.62g/l/h). There was a correlation between ADH and PDC activities and nutrient requirements. For example, KO11-related strain LY01 transformed with plasmids for over-expressing *adh/ pdc*, the latter, required lower levels of supplemental nutrients than the parent strain[34].

**Bioconversion of Cellulose to Ethanol by *Z. mobilis***

The first recombinant strain[35] was engineered to ferment xylose (Fig. 1). Four *E. coli* genes [xylose isomerase (*xylA*), xylulose kinase (*xylB*), transketolase (*tktA*), and transaldolase (*talB*)] were introduced to this strain. *xylA* and *xylB* convert xylose into xylulose-5-phosphate, an important immediate in the pentose phosphate pathway. Xylulose-5-phosphate is next converted to intermediates of the ED pathway by transketolase and transaldolase. The genes were expressed on a plasmid using either the enolase or glyceraldhyde-3-phosphate dehydrogenase promoters from *Z. mobilis*; both of which are strong constitutive promoters. The transformed strain CP4 (pZB5) grew on xylose, and yielded ethanol (86%). The strain also simultaneously fermented glucose and xylose. Xylose uptake depends upon the native glucose permease; *Z. mobilis* does not have active sugar transport systems[36].

For arabinose fermentation[37], a plasmid was constructed with five genes [L-arabinose isomerase (*araA*), L-ribulose kinase (*araB*), L-ribulose-5-phosphate-4-epimerase (*araD*), transketolase (*tktA*) and transaldolase (*talB*)] isolated from *E. coli*. The first three enzymes are responsible for converting

arabinose to xylulose-5-phosphate, which was converted to ED pathway intermediates via transketolase and transaldolase. The resulting transformed strain, ATCC39676 (pZB206), successfully fermented arabinose (25 g/l) to ethanol and displayed a very high yield (98%). But the rate of arabinose fermentation was much lower compared to that observed for the xylose-fermenting strain[36].

By transforming pZB5 into *Z. mobilis* ZM4, resulting strain ZM4 (pZB5) demonstrated higher ethanol tolerance[38] than CP4 derivatives on high concentrations of glucose/xylose (65 g/l) mixtures. However, when sugar concentration was further increased to 75 g/l of each, ZM4 (pZB5) fermentation stalled after the ethanol concentration rose to 67 g/l. Therefore, the maximum ethanol tolerance for ZM4 (pZB5) fermenting a glucose/xylose mixture appears to be approx half that of the wild-type strain fermenting glucose.

*Z. mobilis* strain ATCC39767 carrying pZB5 was able to convert cellulosics to fuel ethanol efficiently[39]. However, this strain is highly sensitive to inhibitors associated with hydrolysates, especially acetic acid[40]. Furthermore, growth on xylose exacerbates the strains sensitivity to acetic acid[41]. Lawford *et al*[40] successfully adapted xylose-fermenting strain ATCC39767 (pZBL4) to tolerate higher concentrations of acetic acid, as well as other inhibitors, by culturing the strain in continuously higher levels of hydrolysate. The continuous culture was run for 149 days and the level of hydrolysate in the medium increased (from 10 to 50%, v/v). Isolates recovered at the end of fermentation demonstrated significantly improved ethanol productivity in the presence of acetic acid.

The adapted strain was subsequently evaluated for converting popular wood hydrolysate to ethanol. The hydrolysate was prepared by steam exploding the poplar wood chips followed by "over-liming" to reduce inhibitor levels. The fermentations were conducted at 34°C and pH 5.5, which represents a compromise between the optimal conditions for the cellulase enzymes and typical culture conditions for *Z. mobilis*. After 7 days, fermentation reached an ethanol concentration of 30g/l (yield 54%) based upon total initial carbohydrates[42].

*Z. mobilis* strain AX101, derived from ATCC39676, fermented both arabinose and xylose and carry seven important heterologous genes. Overall ethanol yield was 0.43 to 0.46g/g with byproducts of xylitol (3.35 g/l), lactic acid (0.21 g/l) and acetic acid (0.84 g/l) from these strains. Lactic acid production was low in this strain compared to previous pentose-utilizing strains, possibly because arabinose fermentation genes were integrated at the site of *ldh* gene[43]. Whereas most reported fermentations using AX101 have employed laboratory media formulations that include expensive yeast extract (5–10 g/l) as a nitrogen source, the strain requires only the addition of CSL (0.5% v/v) to meet its nutritional needs[43]. A new *Z. mobilis* strain 8b achieved good ethanol yield (83%, w/w) and showed a remarkable tolerance[44] to acetic acid (8-16 g/l) at pH 6 and 37°C.

To utilize lignocellulosics, *Ruminococcus albus* β-glucosidase was fused with the signal sequences (53 amino acid) of glucose-fructose oxidoreductase (GFOR) of *Z. mobilis* with an S/T-R-R-X-F-L-K consensus motif. The β-glucosidase gene was efficiently expressed and tag enabled β-glucosidase activity (61%) to be transported through the cytoplasmic membrane of the recombinant strain. The recombinant *Z. mobilis* was able to ferment[12] cellobiose (22 g/l) to produce ethanol (10.7 g/l)

### Exploitation of *Klebsiella oxytoca* and *Erwina chrysanthemi* for Ethanol Production

In addition to *E. coli*, Gram-negative bacteria, *K. oxytoca* and *E. chrysanthemi* were also transformed with the *pet* operon. Though the resulting strains have lower ethanol yields than *E. coli*, significant progress has been made in developing improved *K. oxytoca* strains for converting cellulose to ethanol. *K. oxytoca* is an enteric bacterium found in wood, paper and pulp processing streams. It can grow on a wide variety of sugars including hexoses and pentoses, as well as on cellobiose and cellotriose. These characteristics make the strain important for cellulose fermentations. It can also ferment glucose to a variety of organic acids, neutral products and ethanol through the PFL pathway. *K. oxytoca* M5A1 containing the *pet* operon, produced ethanol (> 90%) of the fermentation products[45]. Expressing the pet operon on a lower copy number plasmid (pLOI555) gave higher ethanol productivity than for the higher copy number plasmid (pLOI297) on contrary to *E. coli* strains[45]. Strain M5A1 (pLOI555) appears to be particularly well suited for fermenting xylose (2 g/l/h during first 24 h) and twice as fast as *E. coli* strain KO11.

The *pet* operon was genetically stabilized in *K. oxytoca* M5A1 by integrating the operon along

with a chloramphenicol acetyl transferase (*cat*) gene at the site of the chromosomal *pfl* gene[46]. Mutant strain (P2) that readily ferments either glucose (100 g/l) or cellobiose (100 g/l) was isolated from chromosomal integrants with higher ethanol yields (44–45g/l) within 48 h. The strain P2 has been tested[47-50] successfully on mixed office paper, sugarcane bagasse, corn fiber and sugar beet pulp. The fermentation of sugarcane bagasse using *K. oxytoca*, P2 produced 38.6 g/l ethanol. Because *K. oxytoca* ferments cellobiose, the cellulase was not supplemented with additional β-glucosidase activity. A two-stage saccharification and fermentation scheme was also developed using raw sugarcane that increased the final ethanol yield (40 g/l), but this process took a total of 13 days. Golias *et al*[51] compared strain P2 and cellobiose-fermenting yeasts for fermenting micro-crystalline cellulose (Sigmacell 50). *K. oxytoca* P2 fermentations were faster (25-50%) than those inoculated with the yeasts, but the final ethanol concentration was limited (37g/l).

Variants of strains P2 have been constructed that express endoglucanase, a component of cellulase mixtures. Engineering strains to produce their own cellulase is one strategy for reducing ethanol production costs. Zhou & Ingram[52] integrated two extracellular endoglucanase genes (CelZ and CelY) from *E. chrysanthemi* into the chromosome of strain P2 with auxiliary transporter gene (*out*) on a plasmid (pCPP2006). The cellulolytic strain was named SZ21[53]. Endoglucanase production measured in glucose-grown cultures of strain SZ21 (pCPP2006) were 20 U/ml, about 1 percent of the activity in commercial cellulase preparations[53]. The strain fermented cellulose (Sigmacell 50) poorly without supplementing with additional cellulase activity. When commercial cellulase was added, SZ21 (pCPP2006) produced higher ethanol (7–16%) than the parental strain. Doran *et al*[50] compared strains *K. oxytoca* P2, *E. coli* KO11 and *E. chrysanthemi* EC16 (pLOI555) for production of ethanol from sugar beet pulp (106 g/l) with simultaneous enzymatic hydrolysis of pectin and cellulose. *E. coli* KO11 fermentations produced more ethanol (40%) than the others.

**Metabolic engineering of *Clostridium cellulolyticum* and *Lactobacillus casei***

*Clostridium cellulolyticum* ATCC 35319, is the best understood cellulolytic mesophilic bacterium[54]. Naturally, high concentrations of pure cellulosic substrates are unfavorable to *C. cellulolyticum*. Under this condition, nutrients or products have been accumulated to toxic levels[55]. The pyruvate overflow suggested (Fig. 2) that the carbon flux through glycolysis was higher than the rate of procession of pyruvate ferredoxin oxidoreductase (PFO) and lactate dehydrogenase (LDH). As a result, catabolic overflow leads to an accumulation of these inhibitory compounds that are directly responsible for the early cessation of growth of the cells. Therefore, *C. cellulolyticum* is not adapted to use a carbon source in excess and the strain is not suitable for fuel ethanol process from cellulosics. To decrease the pyruvate production, an expression system (pMG8) which constitutes the *Z. mobilis pdc* and *adh* genes under the control of a strong ferredoxin gene promoter of *Cellulolyticum pasteurianum* was introduced. The resultant recombinant strain CC-pMG8 was able to grow on cellulose medium with higher specific growth rate (0.049 g/l/h) than that of the parent strain (0.044 g/l/h) and produces two fold more ethanol (20 m*M*). The higher ethanol production was attributed to the utilization of excess intracellular pyruvate[56]. Gold *et al*[57] have also constructed a series of Gram-positive strains for ethanol production. *Z. mobilis pdc* and *adh* genes were introduced into pHP13, a low copy number shuttle vector. *pet* genes were poorly expressed in *B. subtilis* transformants containing pHP13 but no ethanol was produced. Thus, the high copy number, pAMβ1 derived vector pIL253, was used. Later, *pet* genes were placed under the control of *B. subtilis* SP02 phage promoter. Resultant construct pTC207 was transformed into *L. casei* 686, *L. plantarum* 1196, and *B. subtilis*; which had no ethanol production. Although, ADH activity was detected, PDC expression was poor. To increase *pdc* expression in the *Lactococcus* strains, a translational coupling was established with a RBS obtained from the *Lactococcus lactis* expression vector, pMG36e. The translation of *pdc* was designed to be coupled to the translation of 27- amino acid peptide produced from the RBS of pMG3e promoter region, which terminates at the start codon of *pdc* with the sequence ATGA. The final construct pRSG02 was transformed to *L. casei*. In fermentation trials, the *L. casei* harboring pRSG02 produced more than twice the ethanol of parental strain (Table 2). Interestingly, the recombinant strain with cloned *pdc* only produced more ethanol than the strain containing both *pdc* and *adh* with its native ADH activity.

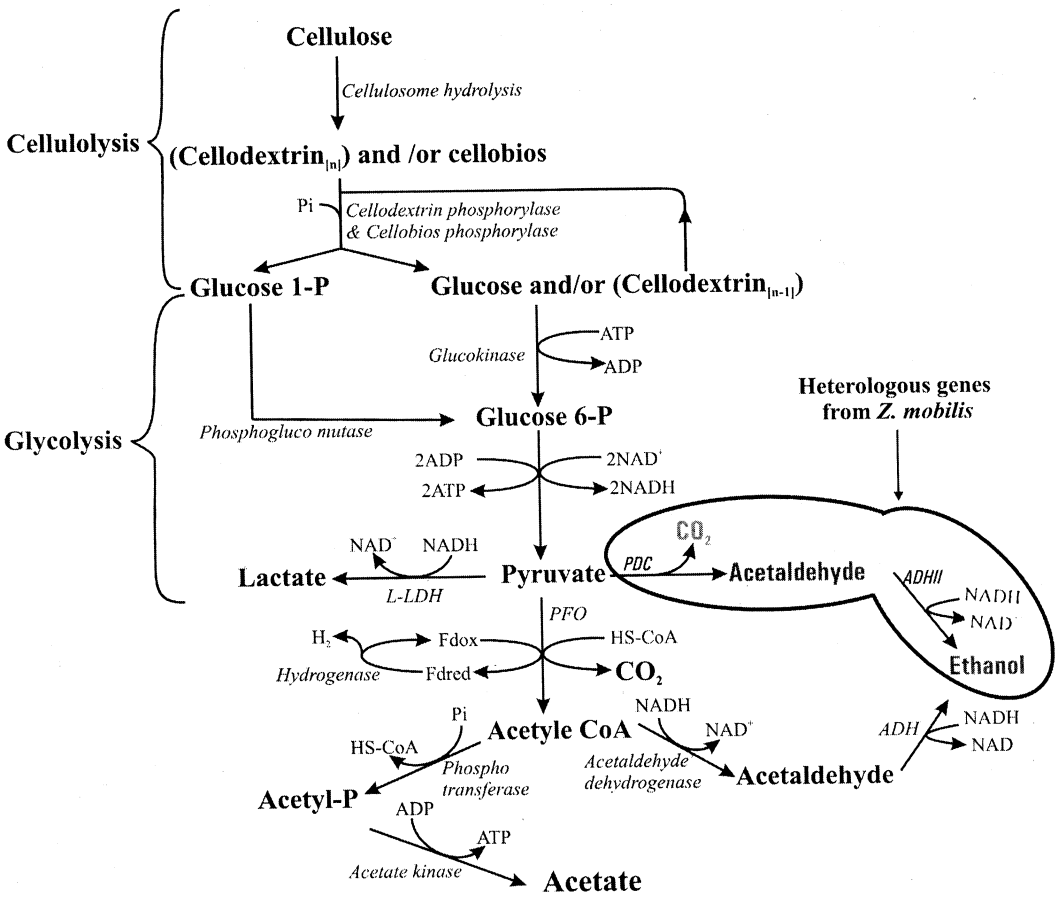


Fig. 2—Bioconversion of cellulose to ethanol by the engineered *C. cellulolyticum*-CCpMG8
P, phosphate; ox, oxidation; red, reduction; HS-CoA, coenzyme A. The *C. cellulolyticum* is a naturally produce ethanol with minimal quantity with excess pyruvate. To direct the excess pyruvate to ethanol the PET genes (marked area) were introduced (Adapted from Guedon *et al.,* 2002)

Table 2—Fuel ethanol production by engineered strains

| Strain | Ethanol g/l | Ethanol yield g/g |
|---|---|---|
| *L. casei (pIL253)* | 1.23 | 0.07 |
| *L. casei* (pRSG01) | 8.71 | 0.16 |
| *L. casei* (pRSG02) | 6.53 | 0.17 |

Substrate concentration 60 g/l (Gold *et al*, 1996)

## Conclusions and Future Prospects

The utilization of bioethanol for transportation has the potential to contribute to a cleaner environment. It is expected that the bioethanol industry will benefit from the efficient utilization of lignocellulosic wastes. Technical achievements in this sector, particularly the satisfactory performance of metabolically engineered microorganisms in pilot scale, have favored optimistic forecasts. Meanwhile, metabolic engineering is addressing further enhancement of microorganism capabilities by adding/modifying traits such as tolerance to ethanol and inhibitors, hydrolysis of cellulose/hemicellulose, thermotolerance, reduced need nutrient supplementation, and improvement of sugars transport. The improvement achieved in the fermentation step with the help of metabolic engineering is just one of the aspects of an integrated process.

Engineered *E.coli* and *K. oxytoca* have sufficient traits for ethanol production from cellulosic substrates. While considering ethanol tolerance and substrate specificity, *Z. mobilis* has desirable traits for fuel ethanol production (Tables 3&4). Gram-positive bacteria *Cellulomonas* and cellulolytic *Bacillus* strains have a higher optimal fermentation temperature than the other bacteria. These organisms can be further engineered to suit for industrial scale. For conversion of lignocellulose to ethanol the use of cellulase, a high cost associated method in enzymatic conversion of cellulose to sugars can be reduced by this direct conversion method. Among the reviewed strains, *Z. mobilis* pZAGFβg performed better and produced (0.4 g/g cellobiose) ethanol with low by-products

Table 3—Parameters used for recombinant host strains to produce ethanol

| Host | Ara | Gal | Glc | Man | Xyl | T, $^oC^a$ | $pH^a$ |
|---|---|---|---|---|---|---|---|
| *E. coli* | + | + | + | + | + | 35 | 6.5 |
| *K. oxytoca* | + | + | + | + | + | 30 | 5.5 |
| *Z. mobilis* | + | - | + | - | + | 30 | 5.5 |
| *C. cellulolyticum* | + | + | + | + | + | 34 | 7.2 |
| *L. casei* | d | d | + | d | d | 37 | 7.0 |

*Ara* Arabinose, *Gal* galactose, *Glc* glucose, *Man* mannose, *Xyl* xylose
d- not determined
[a]Typical culture conditions for single sugar fermenting cultures; conditions are varied for simultaneous saccharification and fermentation

Table 4—Comparison of batch fermentations with cellulose/cellulosic hydrolysate/ sugar mixtures by engineered bacterial strains

| Strain | Host | Sugars | Maximum ethanol g/l | Ethanol yield % | Ethanol productiong l/h | Ref |
|---|---|---|---|---|---|---|
| *E. coli* | KO11 | Xyl 90 | 41.0 | 899 | 0.85 | 31 |
| | | Ara:Gal:Glc:Xyl, 23:11:27:39 | 41.7 | 90 | 0.62 | 21 |
| | FRB5 | Xyl 95 | 41.5 | 90 | 0.59 | 29 |
| | | Ara:Xyl:Glc 15:30:30 | 34.0 | 90 | 0.92 | 29 |
| | LY01 | Xyl 140 | 63.2 | 88 | 0.66 | 31 |
| *K. oxytoca* | M5A1 (pLOI555) | Xyl 100 | 46.0 | 95 | 0.96 | 45 |
| | P2 | Ara:Xyl:Glc 20:40:20 | 34.2 | 84 | 0.35 | 3 |
| *Z. mobilis* | AX101 | Ara:Glc:Xyl 20:40:40 | 42 | 84 | 0.61 | 44 |
| | CP4 (pZB5) | Xyl 160 | 23.0 | 94 | 0.32 | 40 |
| | 29191 (pZAGFβg) | Cellobios 20 | 10.7 | 95 | 0.44 | 12 |
| *C. cellulomonas* | H10 (pMG8) | Cellulose 18 | 20 | 93 | 0.04 | 56 |

such as acetate, succinate and lactate in cellulose medium. Still, a more efficient strain is with chromosomal coded cellulase gene is required.

Both Gram-positive and thermophilic bacteria have unique advantages compared to Gram-negative bacteria. Several reported attempts to express the *pet* operon in Gram-positive bacteria have given disappointing results including very low ethanol yields. The use and improvement in thermophiles is limited by the absence of a genetic system. In the coming years, more efficient bacterial strains will be developed for the direct conversion of lignocellulosics to ethanol.

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