ISSN (Print) 1225-9918
ISSN (Online) 2287-3406
Journal of Life Science 2019 Vol. 29. No. 1. 135~145 DOI : https://doi.org/10.5352/JLS.2019.29.1.135

- Review -

Recent Progress in Strain Development of *Zymomonas mobilis* for Lignocellulosic Ethanol Production

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Received December 5, 2018 / Revised January 3, 2019 / Accepted January 3, 2019

Zymomonas mobilis has been recognized as a potential industrial ethanologen for many decades due to its outstanding fermentation characteristics, including high ethanol tolerance, fast sugar uptake rate, and high theoretical ethanol yield. With the emergence of the postgenomic era and the recent announcement of DuPont's world largest cellulosic ethanol production process, research on this bacterium has become even more important to harness successful application not only for use in the bioethanol process but also in other biochemical processes, which can be included in bio-refinery. As an important industrial microorganism, *Z. mobilis* will likely be exposed to various stressful environments, such as toxic chemicals, including the end-product ethanol and fermentative inhibitory compounds (e.g., furan derivatives, organic acids, and lignin derivatives in pretreatment steps), as well as physical stresses, such as high temperature during large-scale ethanol fermentation. This review focuses on recent information related to the industrial robustness of this bacterium and strain development to improve the ethanol yield and productivity in the lignocellulosic ethanol process. Although several excellent review articles on the strain development of this bacterium have been published, this review aims to fill gaps in the literature by highlighting recent advances in physiological understanding of this bacterium that may aid strain developments and improve the ethanol productivity for lignocellulosic biomass.

Key words : Bacterial physiology, industrial robustness, lignocellulosic ethanol, strain developments, *Zymomonas mobilis*

Introduction

Seeking an alternative to replace the energy use from the conventional hydrocarbon resources has been devoted to considerable public attention over last several decades. Thoughtless use of fossil fuel, its associated air pollution and global warming have been recognized as main causatives. Several candidates have been developed and used such as bioethanol, solar and hydrogen energy. The current technology for major ethanol production is based on the fermentation of sugars derived from biomass including starch or sugar-based crops, agricultural residues. Such biochemical process is related to the manufacture of alcoholic beverages. However, due to cost-competitive process development and ethical dilemma feed use for energy, current industrial ethanol production need further optimization to use cheaper raw materials such as lignocellulosic materials.

Zymomonas mobilis is one of unique bacteria that can produce ethanol as a major product via Enter-doudouroff pathway with the conjunction of ethanol fermentation pathway. This bacterium has been studied for more than several decades for fuel ethanol production via biochemical processes. Since its substrate usage ranges for lignocellulosic ethanol process for this bacterium had been successfully extended via metabolic engineering of pentose utilization, several important genomes of type strains as well as their mutants with system approaches have been analyzed to further understand genetic characteristics [4, 9, 26, 27, 39, 65]. Recent research activities on this bacterium has contributed to the increased potential for further industrial application used in other chemical production [50, 53]. This paper reviews the relatively recent information related to strain development and physiological characteristics associated with this bacterium.

The availability of multiple genome sequence of *Z. mobilis*

After early pioneering attempts to understand physiological characteristics associated with ethanol production,

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this bacterium has been recognized as an alternative ethanol producer [38]. Many fermentation kinetic based studies indicated that this bacterium has several industrial advantages such as generally regarded as safe (GRAS) bacterium, displaying high ethanol tolerance up to 16% (v/v), higher specific glucose uptake rate leading to higher ethanol productivities and less energy use for biomass yield relative to *Saccharomyces cerevisiae* which can allow to produce ethanol close to the maximum theoretical yield [37, 38]. Such advantages on this bacterium heightened the interests to understand genetic information associated with ethanol production characteristics. Since the first genome analysis and annotation have been announced by Seo *et al.* [39], other type strains of the genome information as well as their mutants have been analyzed as shown in Table 1. In general the genome size of all *Z. mobilis* strains were in the range of 2.1 to 2.22 Mb with two to eight plasmids contained which is only half of the genome size of *E. coli* (4.8Mb). By virtue of the multiple genome availabilities of this bacterium, system based research using transcriptomic and proteomic analyses allows the further understanding of global responsible genes associated with particular genetic traits as well as the genes involved in fermentation associated stress conditions [6, 16, 22, 55, 59, 61].

Ethanol production from lignocellulosic materials via substrate usage extension of *Z. mobilis*

Fuel grade ethanol can be readily produced by fermentation of starch hydrolysate or hexose sugar from crop-based resource such molasses, sucrose-containing juices from sugar cane or sugar beets, potatoes, fruits and grains including maize and wheat etc. However, such raw materials used in ethanol fermentation step account for about 55-60% of the production cost [48]. Therefore, for the cost effective ethanol production have been needed the use of cheaper raw materials such as lignocellulosic biomass. Such cheaper raw materials are the most abundant renewable energy source and available from various industries including agricultural, forestry and food wastes. Although their compositions vary, generally composed of 45-50% cellulose, 25-30% hemicellulose and 15-25% lignin. The bioconversion process of lignocellulose to ethanol in general needs the saccharification of both the hemicellulose and cellulose fraction. Hemicellulose are highly branched non-crystalline hetero-polysaccharides comprising of pentose, hexose and uronic acid. Wild type strains of *Z. mobilis* can only utilize glucose, fructose and sucrose as carbon source, but not abundant pentose sugars such as xylose and arabinose derived from lignocellulosic materials [37]. To overcome such limitation, bioethanol research on this bacterium as an alternative has been carried out for one of main challenges on substrate usage extension via metabolic engineering. Despite several early pioneering challenges on this mission by several authors [7, 11], their work was not successful due to the lack of genome information for metabolic engineering. However, with continuous efforts NREL group announced the successful development of a xylose utilizing plasmid bearing strain of CP4 (pZB5) via heterogeneous genes from *E. coli* [62]. Since then, various pentose sugar fermenting strains via metabolic engineering have been reported for lab- or industrial- scale ethanol production as shown in Table 2.

Since then many research to evaluate the recombinant strains via fermentation kinetics, ethanol production yield and productivities using various lignocellulose feedstock have been carried out. The most all recombinant xylose-fermenting *Z. mobilis* showed decreased in the yields and volu-

Table 1. The list of *Z. mobilis* strains genome sequenced

Strains	Accession No.	Status	Genome size (Mb)	No. of predicted genes	Plasmid	References
ZM4 (ATCC31821)	NC_006526.2	Complete	2,06	1,830	5	[39, 56]
ATCC29191	NC_018145.1	Complete	2,0	1,761	3	[9]
ATCC29192	NC_015709.1	Complete	1.99	1,763	2	[26]
ATCC10998	NC_017262.1	Complete	2.02	1,820	6	[36]
ZM401 (ATCC32822)	AMSR00000000.1	Draft genome	2.04	-	In scaffolds	[65]
ZM481 (ATCC32823)	LSFP00000000.1	Draft genome	2.20	-	In scaffolds	[66]
NRRL B-12526	NZ_CP003709.1	Complete	2.01	1,946	4	-
CP4 (NRRLB-14203)	NZ_CP003715.1	Complete	2.01	1,918	5	[28]
NRRL B-1960	CP021053	Complete	2.05	1,835	2	[4]
NCIMB 11163	CP001722.1	Complete	2.12	1,947	3	[27]

Table 2. The list of heterologous genes expressed in *Z. mobilis* to broaden its substrate usages for ethanol production

Strains	Genes	Functions	Source	Goal	Ethanol yield (g g^{-1})	Ref.
CP4 (pZB5)	*xylA, xylB, talB, tktA*	xylose isomerase, xylulokinase, transaldolase, transketolase	*E. coli*	Xylose utilization	0.44	[62]
ATCC39676 (pZB206)	*araA, araB, araD, talB, tktA*	Arabinose isomerase, ribulokinase, ribulose-5-phosphate-4-epimerase, transaldolase, transketolase	*E. coli*	Arabinose utilization	0.50	[8]
CP4 (pZY228)	*xylA, xylB; tktA*	Xylose isomerase, xylulokinase; transketolase	*Klebsiella pneumonia; E. coli*	Xylose utilization	ND	[11]
CP4 (pZY228, pZY557tal)	*xylA, xylB, talB, tktA*	xylose isomerase, xylulokinase, transaldolase, transketolase	*E. coli*	Xylose utilization	0.29	[7]
ZM4 (pKLD3, pKLD4,5)	*xylA, xylB, talB, tktA, XylE*	xylose isomerase, xylulokinase, transaldolase, transketolase, xylose transporter	*E. coli*	Xylose utilization	0.40	[10]
8b	*xylA, xylB, talB, tktA, araA, araB, araD, talB, tktA*	xylose isomerase, xylulokinase, transaldolase, transketolase, Arabinose isomerase, ribulokinase, ribulose-5-phosphate-4-epimerase, transaldolase, transketolase	*E. coli*	Xylose and arabinose utilization	0.4	[34]
CP4HYMX3	*xylA, xylB, tktA, talB, yfdZ, metB; Pfu-sHSP*	xylose isomerase, xylulokinase, transaldolase, transketolase, aminotransferase, cystathionine gamma-synthase, heat shock protein	*E. coli; Pyrococcus furious*	Xylose utilization, Vitamin self-use, low nutritional requirement, high temperature resistance	0.41	[63]

metric productivities of ethanol. For an example, *Z. mobilis* ZM4(pZB5) are significantly reduced in ethanol yield, xylose uptake rate and ethanol productivity due to the fermentation inhibitory compounds derived during the pretreatment step as compared to those from artificial lignocellulosic hydrolysates as shown in Table 3. Such inhibitory compounds categorized based on their origins. Sugar derivatives include furfural, hydroxyl methyl furfural (HMF) and levulinic acid, organic acids including acetic acid and formic acid, and lignin derivatives including vanillin, vanillic acid and syringaldehyde. Besides such inhibitory toxic compounds, ethanol it-self and high temperature during fermentation could also result in negative effects on cell growth and metabolism for ethanol production by this bacterium. Owing to the recent multiple genome availability and strain development strategies for this bacterium, many research to improve its robustness with physiological understanding of various mutant strains against such inhibitory compounds will be further described in following sections.

Alcohol tolerance mechanism of *Z. mobilis*

During ethanol fermentation, all ethanol producing strains

Table 3. Effect of lignocellulosic inhibitory compounds on specific rates of xylose uptake, ethanol production, and ethanol yield of *Z. mobilis* ZM4 (pZB5) at 30℃ and initial pH 6 investigated by Kim *et al.* [25]

Compounds	Compounds concentration (g l^{-1})	Specific xylose utilization rate (g (g h)$^{-1}$)	Specific ethanol production rate (g (g h)$^{-1}$)	Ethanol yield (g g^{-1})
Control		1.58	0.63	0.40
Sodium acetate	10.9	1.15	0.46	0.40
Furfural	0.3	1.40	0.53	0.38
HMF[a]	0.9	1.36	0.54	0.40
Vanillin	0.043	1.24	0.49	0.40
Vanillic acid	0.084	1.57	0.63	0.40
Syringaldehyde	0.13	1.24	0.50	0.40

[a]: HMF abbreviates hydroxymethylfurfural.

are progressively faced to ethanol toxicity which decreases the rate of sugars being converted into ethanol. Previous studies indicated that this bacterium started to express stress response in the presence of more than 60 g l^{-1} ethanol where glucose uptake rate and cell biomass yields are decreased, although ethanol tolerance in this bacterium tolerate up to 16%(w/v) ethanol [37]. This limits the final concentrations of ethanol in the fermentation step, which leads to the increase in overall cost of ethanol production. Several papers describe the mechanism of ethanol toxicities in this bacterium as well as in other ethanologens [5, 20, 21, 33, 60]. The accumulated ethanol surrounding the medium causes two toxic effects resulting in the irreversible fates of bacteria. One physiological response is an increase in cell-envelope permeability. As alcohols accumulated inside of cell membrane and this disorganizes their structure, which results in a loss of ions, metabolites, changes the intracellular pH and membrane electrical potential, and eventually leads to cell death. The other is several decoding machineries involved in transcription and translation steps are more likely susceptible to coordinate the central cell functionality [15].

Recently Wang et al [45] reported a recombinant xylose fermenting strain enhancing free fatty acid biosynthesis for ethanol tolerance increase via heterologous expression of fatty acid synthesis pathway including thioesterase (TesA) and the wax ester synthase/acyl-CoA–diacyl glycerol acyltransferase (WS/DGAT) from *Acinetobacter baylyi* together with fatty acyl-CoA reductases from jojoba (FAR). The approach attempted to make more stable cell envelope for this bacterium under high ethanol concentrations. In particular the authors demonstrated that this strain can be used in very high gravity ethanol fermentation process containing high concentration of sugar more than 250 g l^{-1} which allows to exposure to a high ethanol concentration.

Previously believed that ethanol tolerance mechanism have been supposed to be accumulation of hopanoids in cell envelope in this bacterium which is enable to maintain membrane stability and viscosity like sterols in eukaryotes [19-21, 40]. However other group found no indication of accumulated hopanoids as a real responsible mechanism for ethanol tolerance [18, 35, 55]. In this respect to understand global response involved in central decoding machineries on the ethanol tolerance mechanism in this bacterium, He *et al.* [16] investigated transcriptome response to identify the genes expression required for tolerance to ethanol. The authors found that 127 genes were differentially expressed in response to ethanol which were involved in a wide range of cellular processes including carbohydrate metabolism, cell wall/membrane biogenesis, respiratory chain, terpenoid biosynthesis, DNA replication, DNA recombination, DNA repair, transport, transcriptional regulation, some universal stress response, etc. They concluded that major response were associated with chaperons and transcriptional regulators. The results were also agree to integrated omics studies by other group [55]. In this respect, Tan *et al.* [43] have also studied to improve its genetic traits of ethanol tolerance via the random mutagenesis of global transcription factor RpoD protein which has been known as a sigma factor. Their results demonstrate that the RpoD mutation can enhance ethanol tolerance in this bacterium. All mutants isolated from error-prone PCR libraries of RpoD gene showed significant growth improvement in the presence of ethanol stress as compared to the control strain. Therefore, ethanol productivities can be improved via global transcriptional transcription machinery engineering. However, the sterol-like hopanoids was not identified as a major response or difference under the stress conditions. In this respect, hopanoids synthesis pathway in this bacterium requires further investigation to understand ethanol stress concerns.

Responsive small RNAs (sRNA) to understand how this bacterium regulate their specific metabolic pathway under ethanol stress conditions have been also investigated [6]. Fifteen novel sRNAs haven been identified from this bacterium. Among them, three of sRNAs (Zms2, Zms6, and Zms18) were differentially expressed under 5% ethanol stress conditions although their functionalities need to be further investigated.

Osmotolerance

Previous investigation suggested that *Z. mobilis* can tolerated up to 400 g l^{-1} carbohydrate which is one of unique characteristic of this ethanol producing bacterium [37]. *Z. mobilis* also produces sorbitol as a major by-product when it is grown in sucrose or mixtures of glucose and fructose, although sorbitol are formed as minor product as glucose or fructose alone respectively used as substrates for this bacterium [3, 30, 32]. The formation of sorbitol resulted from the *in vivo* inhibition of fructokinase by glucose. Subsequently, fructose is accumulated and then converted into sorbitol by the action of glucose-fructose oxidoreductase (GFOR) [32]. The addition of sorbitol into culture medium promotes the growth of *Z. mobilis* when grown in a high-sugar medium

suggested that sorbitol protected cells from harmful effects caused by high osmotic pressures.

In addition to the osmoprotective function by sorbitol, its protective function associated with heat and ethanol stresses have also been investigated [41]. In this respect, *gfo* gene encoded for GFOR was inactivated by gene disruption technique, and the strain was designated as *Z. mobilis* Δ*gfo*. The Δ*gfo* strain show the reduction of cell growth and ethanol production under osmotic stress as well as under heat and ethanol stresses indicating that sorbitol not only promoted cell growth but also increased the fermentation capability of *Z. mobilis* under heat and ethanol stresses conditions tested.

Temperature tolerance

Ethanol fermentation process has been known as one of exothermic reaction [44] that progressively increases heat stress to ethanologens which reduces their growth or viability during the ethanol fermentation process. Therefore if ethanologens have abilities tolerate to over critical high temperatures, it can introduce several advantages into the process such as reduction in cooling cost, saving of enzyme cost in simultaneous saccharification and fermentation (SSF) or prevention of contamination from unfavorable microbes[33, 44] . With this aim several research on developing thermotolerant strain of *Z. mobilis* have been investigated using gene disruption technique, heterologous expression and conventional chemical mutagenesis followed by acclimated high temperature condition [5, 33, 41, 45, 63]. Charoensuk *et al.* [5] investigated transposon mutagenesis to identify essential genes associated with thermotolerant mechanisms for survival at a critical high temperature at 39℃ using *Z. mobilis* TISTR 548 which is a natural thermotolerant stain isolated via chemical mutagenesis with acclimated high temperature conditions. The authors found that thermotolerant of this bacterium were related to one of heat shock protein called *degP*. The authors conclude that thermo-tolerance by the heat shock protein was also associated with Reactive Oxygen Species (ROS) indicating oxidative stress is also related to thermal tolerance, and membrane stabilization associated with fatty acid synthesis pathway.

In the respect of heat shock protein involvement, heterologous expression of small heat shock protein gene (*Pfu-sHSP*) from *Pyrococcus furious* was also investigated to increase the heat-tolerance in this bacterium [45, 63]. The recombinant strain designated as HYMX demonstrated the high tolerance to high temperature.

In other respect to membrane stabilization, Wang *et al.* [45] investigated heterologous expression of fatty acid synthesis pathway including thioesterase (TesA) and the wax ester synthase/acyl-CoA–diacyl glycerol acyltransferase (WS/DGAT) from *Acinetobacter baylyi* together with fatty acyl-CoA reductases from jojoba (FAR). The recombinant strain have been demonstrated multiple tolerance including temperature associated stress existed in very high gravity (VHG) ethanol fermentation process.

Acetate tolerance

Acetic acid is one of ubiquitous fermentative inhibitory compounds for ethanologen. This compound is derived during pretreatment step for the destruction of lignocellulosic feedstocks. Acetic acid is a weak acid with a pKa of 4.75 and is considered as an anti-microbial compound in the food and beverage industries. This chemical dissociates into an anionic species and a proton with the degree of dissociation dependent on the pH. Inhibitory concentrations of this compound for this bacterium have been investigated by several previous studies which reveal that around 10.9 g 1^{-1} at pH 5.0 [25, 31]. The resistance against this compound in particular have been attention to develop acetate resistance strains via chemical mutagenesis such as N-methyl-N′-nitro-N-nitrosoguanidine followed by acclimated acetate stress conditions [12, 23, 31, 51]. Acetate tolerance associated genes have also been elucidated via genomic, transcriptomic and proteomic analyses [52, 54]. The authors found that sodium-proton antiporter gene *nha*A (ZMO0119) is associated with sodium acetate tolerance. Yang *et al.* [51] also investigated gene related to acetate toxicity using a xylose/arabinose fermenting strain 8b. Their transcriptome results also suggested that the presence of acetate caused the genes related to biosynthesis, the flagellar system, and glycolysis to be downregulated, and genes related to stress responses and energy metabolism to be upregulated. Among the several gene candidates, the gene knockout mutant of TonB-dependent receptor (ZMO0128) is associate with acetate tolerance, although its function needs to be further understood.

Furfural, hydroxymethylfurfural and phenolic compounds associated tolerance

Furfural and hydroxymethylfurfural (HMF) formed by dehydration of pentoses and hexoses, and phenolic compounds produced from the partial breakdown of lignin components during the harsh pretreatment process, are another

major inhibitors of lignocelluloses hydrolysates [13, 17, 47, 59]. As a result, ethanol fermentation of *Z. mobilis* is significantly affected by furfural and HMF for lignocellulosic ethanol process. It has been reported that this bacterium has an ability to reduce furfural or HMF into corresponding furfurly alcohol or HMF alcohol, suggesting that the bacterium has native alcohol dehydrogenases or aldo-keto reductases [13]. In this respect, Wang *et al.* [47] identified the gene encoding a NADPH-dependent alcohol dehydrogenase (ZMO 1771) which is related to the conversion of the two furan aldehyde to less toxic phenolic alcohol. In this respect, the authors developed a strain overexpressing the alcohol dehydrogenase together with co-expression of the transhydrogenase gene, *udh*A for its cofactor generation[47]. The recombinant strain showed a significant improvement against the furan aldehyde inhibitors found in lignocellulosic hydrolysate. In the similar respect, aldose reductase gene ZMO 0976 from *Z. mobilis* ZM4 has been reported to be responsible for converting furfural and hydroxymethyl furfural (HMF) to less toxic furfuryl alcohol and HMF alcohol respectively [1].

Stress resistance is well known to be the result of several gene regulation by transcriptional factors. In this respect to increase resistance to furfural and HMF in this bacterium, Tan et al [43] reported a strategy by regulating the transcriptional level via engineering global transcription factor sigma gene (σ^{70}, RpoD). The three *rpo*D mutant strains isolated via error-prone PCR showed the improvement of furfural and HMF tolerance together with ethanol production in this bacterium. In the similar respect, Yang et al [57]investigated the function of a global regulator *hfq* (ZMO0347), as an RNA binding protein, using insertional mutation technique. The authors found that the gene is involved in coordinating regulatory responses to multiple stresses including aldehyde stress, although the authors could not conclude how *hfq* regulates and controls the gene expression response.

Gu *et al.* [14] demonstrated that the physiological response of this bacterium in comparison with traditional yeast strain against to the twelve typical phenolic compounds derived from the part of lignin from lignocellulose. *Z. mobilis* ZM4 showed nearly the same tolerance to the phenolic aldehydes with *S. cerevisiae* DQ1, but the strain showed the stronger tolerance to the phenolic acids originated from corncob hydrolysates including 2-furoic acid, p-hydroxybenzoic acid, p-coumaric acid, vanillic acid, ferulic acid, and syringic acid. The results indicated that this bacterium has the capability for *in situ* detoxification of phenolic aldehydes together with lipopolysaccharide on the cell outer membrane of this bacterium providing the permeable barrier for such phenolic acids. In this respect, Yi *et al.* [59] also elucidated tolerance and detoxification mechanism of phenolic aldehyde inhibitors via transcriptome analysis. The authors found that 272 genes showed twofold greater expressions than non-treated controls, and 36 gene clusters in response to these phenolic aldehydes were identified. Several reductases encoded by glutamate synthase (ZMO1116), alcohol dehydrogenase (ZMO1696), and NADH oxidase (ZMO1885) were found to play the key roles in reducing phenolic aldehydes into the corresponding phenolic alcohols confirmed by overexpression of respective genes (ZMO1116, ZMO1696 and ZMO 1885) in *Z. mobilis* ZM4.

Salt tolerance

Inorganic salts from lignocellulosic materials can also be introduced during the acid or alkali pretreatment processes as well as subsequent neutralization or conditioning steps for saccharification and fermentation. Recently wide range of inorganic chemicals found in biomass feedstock hydrolysate have been tested using an industrial strain of *Z. mobilis* 8b by Franden et al [13]. Among the cations, Ca^{++} and Na^{+} ions showed high cell toxicity to *Z. mobilis*. In this regard, Na^{++} tolerant strain of ZMT2 have been isolated by Wang et al [46]. The authors demonstrated that the strain of salt tolerance against Na^{++} can be achieved via *him*A gene (ZMO1122 encoding integration host factor) disruption via Tn5-based transposon mutagenesis. Among the isolates, the mutant strain ZMT2 with improved salt tolerance phenotype was obtained in the presence of high amount of Na^{++}. The strain ZMT2 was further confirmed to exhibit better fermentation performance under NaCl stress than the wild type strain of ZM4.

The other sodium salt tolerance gene encoding sodium-proton antiport gene *nh*aA was also identified by via microarray analysis, and further confirmed by the gene overexpression studies using the mutant strain of acetate resistance ($ZM4Ac^{R}$) isolated via the chemical mutagenesis followed by under high sodium acetate condition. Comparing the fermentation characteristics of $ZM4Ac^{R}$ in the presence of sodium acetate and NaCl, the glucose consumption and ethanol production by this mutant strain was higher than those of the parental strain ZM4. Although the $ZM4Ac^{R}$ strain was originally selected for sodium acetate tolerance,

this strain also had enhanced tolerance to NaCl [52].

Cell flocculation contribution to the multiple tolerance against various fermentation inhibitors

The cell to cell attachment phenomena called cell flocculation is one of advantage characteristics for ethanol producing microorganism used in the large-scale ethanol production process. A simple and cost-effective way to separate cell biomass from fermentation broth and high volumetric productivities derived from high cell density characteristics provides potential economic feasibility for lignocellulosic ethanol production. In this respect, the flocculent strain *Z. mobilis* ZM401 strain have been isolated via chemical mutagenesis as shown in Fig 1. In addition, its fermentation characteristics and genomic information have been investigated by several studies [22, 29, 49, 64, 65]. Xia *et al.* [64] demonstrated that this flocculating strain has superior ethanol fermentation characteristics than those from planktonic cell, especially in the presence of acetic acid and vanillin derived from lignocellulose. With this regard, to understand the cell flocculation mechanism in *Z. mobilis*, Jeon et al [22] carried out expression microarray studies. The authors found that the mutation in the secondary messenger, c-di-GMP regulating bifunctional enzyme, c-di-guanyl cyclase/phosphodiesterase (ZMO1055) was one of causatives for cell flocculation in this bacterium. The authors hypothesized that high intracellular concentration of c-di-GMP caused by mutated phosphodiesterase domain may result in the increase in bacterial cellulose synthesis metabolism and decrease in flagellar synthesis metabolism as shown in Fig 2. In this respect, Xia et al [49] demonstrated that cell flocculation matrix used by *Z. mobilis* is bacterial cellulose by the gene disruption of cellulose synthase gene encoded by ZMO1083. Therefore, the self-immobilized strain *Z. mobilis* would be one of promising

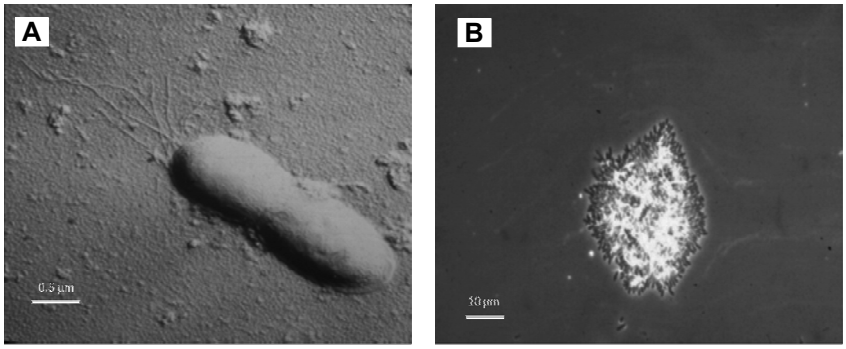

Fig 1. A) Transmission electron microscopic image of flagellated cells of the wild type strain Z. mobilis ZM4; B) flocculent cells of the mutant strain ZM401 image stained by calcofluor white, the source adapted from Jeon *et al.* [22].

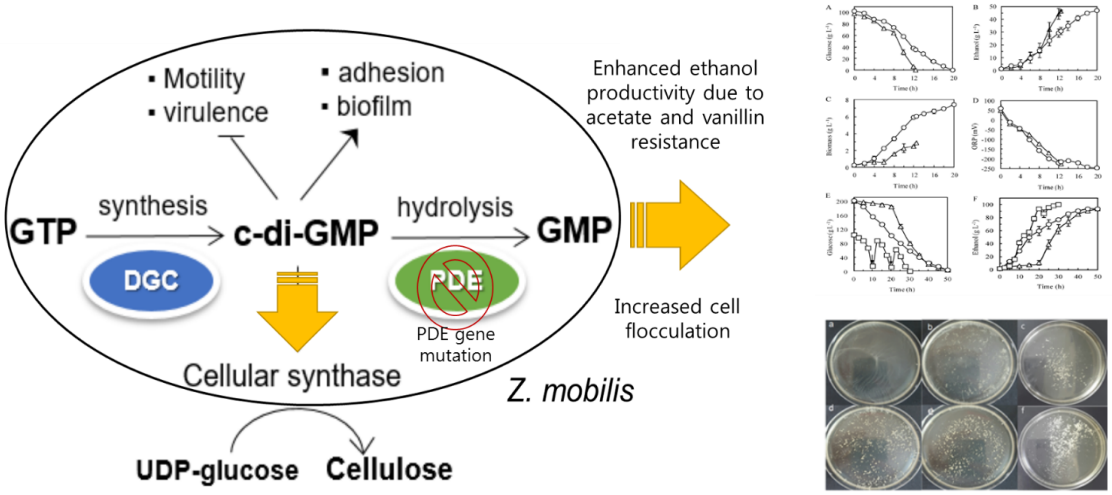

Fig 2. Schematic diagram of cell flocculating mechanims in *Z. mobilis* ZM401 caused by a single point mutation in phophodiesterase domain which allow to increase intracellular level of c-di-GMP. The increased intracellular c-di-GMP subsequently results in increase in cellulose synthsis and decrease in motility. The increased cellulose synthesis metabolism with increased cell flocculation phenotype finally contributed to the enhanced volumatric ethanol productivity due to the acetate and vanillin resistance. DGC: Diguanyl cylase; PDE: Phophodiesterase.

host to be engineered for biofuel ethanol production from lignocellulosic biomass with increased in the tolerance to toxic compounds and high volumetric ethanol production.

Decrement in nutritional requirement

The nascent cellulosic ethanol industry is more likely to be struggle to cost-competitive against corn, sugar cane ethanol and gasoline. Therefore, simple nutrient requirement for industrial ethanologen would provide substantial benefits for economically feasible ethanol production. In particular lignocellulosic feedstocks have low contents of nitrogen source which requires for the ethanologen to grow. Corn steep liquor is a popular industrial nitrogen sources that serves as a source of nitrogen and vitamin. In this respect, Kremer *et al.* [24] hypothesized that this bacterium may have nitrogen fixation capacity since most of the strains *Z. mobilis* genomes encode a nitrogenase, which is required for the N_2 fixation allowing to use N_2 as a nitrogen source through the nitrogen fixation pathway. The authors carried out nitrogen fixation capacity with N_2 enriched with the heavy isotope ^{15}N in the fermentation studies. They found that the synthesized protein from *Z. mobilis* culture contained ^{15}N. Therefore, this demonstrated that N_2 can be used as nitrogen source for *Z. mobilis* which could save a cellulosic ethanol

Table 4. List of genes from *Z. mobilis* involved in stress responds confirmed by various techniques

Gene	Annotation	Host	Techniques	Phenotypes	Ref.
ZZ6_0980	Serine protease DegP	TISTR 548	Transposon insertion	Essential thermo-tolerant gene allowing to survive at 39℃	[5]
ZMO1083	Bacterial cellulose synthase A	ZM401	Gene disruption	Self-flocculation and development of cellulose fibrils increased in multiple tolerance against furfural, HMF, acetic acid etc.	[58]
ZMO1649	Gluconolactonase	ZM4	Gene disruption	Reduced in ethanol productivity in the presence of high glucose concentration (>200 g l^{-1}) under aerobic or fermentative conditions allowing the multiple tolerance against lignocellulosic derivative compounds such as furfural, HMF and acetate etc.	[2]
ZMO0976	NADH- dependent furfural reductase	*E. coli*	Heterologous expression	Involved in reducing the formation of xylitol, furfural and HMF	[1]
ZMO1122 (*himA*)	Integration host factor	ZM4	Transposon insertion	Essential salt tolerant gene allowing to grow up to 2% NaCl	[46]
ZMO0119 (*nha*A)	Sodium-proton antiport gene	$ZM4Ac^R$	Overexpression	Acetate and sodium tolerance	[52]
ZMO1623 (*rpo*D)	RNA polymerase sigma factor	ZM4	Error-prone PCR	Furfural tolerance	[43]
ZMO1771	NADPH-dependent alcohol dehydrogenase	ZM4	Overexpression	Furfural, HMF tolerance	[47]
ZMO0128	TonB-dependent receptor	8b	Gene disruption	Ammonium acetate tolerance (15 g l^{-1})	[51]
ZMO0689	Glucose-fructose oxidoreductase	TISTR548	Gene disruption	Sorbitol production associated with osmotolerance, ethanol tolerance and high temperature tolerance	[41]
ZMO1116, ZMO1696, and ZMO1885	Glutamate synthase, alcohol dehydrogenase, and NADH oxidase	8b	Overexpression	4-hydroxybenzaldyhyde, vanillin	[59]
ZM01055 and ZMO1083	di-guanyl cyclase/phosphodiesterase and cellulose synthase A	ZM401	Microarray and gene disruption	Cell flocculation enhancement associated with acetic acid and vanillin tolerance	[22, 49]

production facility more than $1 million per year [24].

Future prospects

For an economically sensible lignocellulosic ethanol production using *Z. mobilis*, large amount of literature have been primary focused on increasing its industrial robustness against fermentation inhibitory compounds and physical stress conditions which will be more likely to affect in large scale ethanol production cost. Owing to successful metabolic engineering for substrate usage range for pentose sugars derived from lignocellulosic materials and multiple genome information availability, some responsible genes related to physiological behavior of *Z. mobilis* under such environmental stress conditions have been elucidated which is summarized in Table 4. This valuable information would lead to develop further efficient strains improvement for lignocellulosic ethanol production. However, to be cost competitive ethanol production, this strain needs to be further engineered for consolidated bioprocessing in which includes cellulase production, substrate hydrolysis and fermentation are achieved in a single step with only one microorganism. In addition, the recent strain development on this bacterium related to industrial robustness would attract much attention not only in the field of ethanol strain engineering for this bacterium, but also in other chemical production as a possible solution for bio-refinery facilities.

Acknowledgement

This work was supported by the Pukyong National University Research fund in 2017 (CD20171180).

References

1. Agrawal, M. and Chen, R. R. 2011. Discovery and characterization of a xylose reductase from *Zymomonas mobilis* ZM4. *Biotechnol. Lett.* **33,** 2127-2133.
2. Alvin, A., Kim, J., Jeong, G. T., Tsang, Y. F., Kwon, E. E. and Neilan, B. A., *et al.* 2017. Industrial robustness linked to the gluconolactonase from *Zymomonas mobilis*. *Appl. Microbiol. Biotechnol.* **101,** 5089-5099.
3. Barrow, K. D., Collins, J .G., Leight, D. A., Rogers, P. L. and Warr, R. G. 1984. Sorbitol production by *Zymomonas mobilis*. *Appl. Microbiol. Biotechnol.* **20,** 225-232.
4. Chacon-Vargas, K., Chirino, A. A., Davis, M. M., Debler, S. A., Haimer, W. R. and Wilbur, J. J., *et al.* 2017. Genome Sequence of *Zymomonas mobilis subsp. mobilis* NRRL B-1960. *Genome Announc.* **5**, e00562-17.
5. Charoensuk, K., Sakurada, T., Tokiyama, A., Murata, M., Kosaka, T. and Thanonkeo, P., *et al.* 2017. Thermotolerant genes essential for survival at a critical high temperature in thermotolerant ethanologenic *Zymomonas mobilis* TISTR 548. *Biotechnol. Biofuels.* **10,** 204.
6. Cho, S. H., Lei, R., Henninger, T. D. and Contreras, L. M. 2014. Discovery of ethanol-responsive small RNAs in *Zymomonas mobilis*. *Appl. Environ. Microbiol.* **80,** 4189-4198.
7. De Graaf, A. A., Striegel, K., Wittig, R. M., Laufer, B., Schmitz, G. and Wiechert, W., *et al.* 1999. Metabolic state of *Zymomonas mobilis* in glucose-, fructose-, and xylose-fed continuous cultures as analysed by ^{13}C- and ^{31}P-NMR spectroscopy. *Arch. Microbiol.* **171,** 371-385.
8. Deanda, K., Zhang, M., Eddy, C. and Picataggio, S. 1996. Development of an arabinose-fermenting *Zymomonas mobilis* strain by metabolic pathway engineering. *Appl. Environ. Microbiol.* **62,** 4465-4470.
9. Desiniotis, A., Kouvelis, V. N., Davenport, K., Bruce, D., Detter, C. and Tapia, R., *et al.* 2012. Complete genome sequence of the ethanol-producing *Zymomonas mobilis subsp. mobilis* centrotype ATCC 29191. *J. Bacteriol.* **194,** 5966-5967.
10. Dunn, K. L. and Rao, C. V. 2014. Expression of a xylose-specific transporter improves ethanol production by metabolically engineered *Zymomonas mobilis*. *Appl. Microbiol. Biotechnol.* **98,** 6897-6905.
11. Feldmann, S. D., Sahm, H. and Sprenger, G. A. 1992. Pentose metabolism in *Zymomonas mobilis* wild-type and recombinant strains. *Appl. Microbiol. Biotechnol.* **38,** 354-361.
12. Franden, M. A., Pienkos, P. T. and Zhang, M. 2009. Development of a high-throughput method to evaluate the impact of inhibitory compounds from lignocellulosic hydrolysates on the growth of *Zymomonas mobilis*. *J. Biotechnol.* **144,** 259-267.
13. Franden, M. A., Pilath, H. M., Mohagheghi, A., Pienkos, P. T. and Zhang, M. 2013. Inhibition of growth of Zymomonas mobilis by model compounds found in lignocellulosic hydrolysates. *Biotechnol. Biofuels.* **6,** 99.
14. Gu, H., Zhang, J. and Bao, J. 2015. High tolerance and physiological mechanism of *Zymomonas mobilis* to phenolic inhibitors in ethanol fermentation of corncob residue. *Biotechnol. Bioengin.* **112,** 1770-1782.
15. Haft, R. J., Keating, D. H., Schwaegler, T., Schwalbach, M. S., Vinokur, J. and Tremaine, M., *et al.* 2014. Correcting direct effects of ethanol on translation and transcription machinery confers ethanol tolerance in bacteria. *Proc. Natl. Acad. Sci. USA.* **111,** E2576-2585.
16. He, M. X., Wu, B., Shui, Z. X., Hu, Q. C., Wang, W. G. and Tan, F. R., *et al.* 2012. Transcriptome profiling of *Zymomonas mobilis* under ethanol stress. *Biotechnol. Biofuels.* **5,** 75.
17. He, M. X., Wu, B., Shui, Z. X., Hu, Q. C., Wang, W. G. and Tan, F. R. 2012. Transcriptome profiling of *Zymomonas mobilis* under furfural stress. *Appl. Microbiol. Biotechnol.* **95,** 189-199.
18. Hermans, M. A., Neuss, B. and Sahm, H. 1991. Content and composition of hopanoids in *Zymomonas mobilis* under various growth conditions. *J. Bacteriol.* **173,** 5592-5595.
19. Horbach, S., Neuss, B. and Sahm, H. 1991. Effect of azasqua-

lene on hopanoid biosynthesis and ethanol tolerance of *Zymomonas mobilis*. *FEMS Microbiol. Lett.* **79,** 347-350.
20. Ingram, L. O. 1986. Microbial tolerance to alcohols: role of the cell membrane. *Trends Biotechnol.* **4,** 40-44.
21. Ingram, L. O. 1989. Ethanol tolerance in bacteria. *Crit. Rev. Biotechnol.* **9,** 305-319.
22. Jeon, Y. J., Xun, Z., Su, P. and Rogers, P. L. 2012. Genome-wide transcriptomic analysis of a flocculent strain of *Zymomonas mobilis*. *Appl. Microbiol. Biotechnol.* **93,** 2513-2518.
23. Joachimsthal, E., Haggett, K. D., Jang, J. H. and Rogers, P. L. 1998. A mutant of *Zymomonas mobilis* ZM4 capable of ethanol production from glucose in the presence of high acetate concentrations. *Biotechnol. Lett.* **20**.
24. Kremer, T. A., LaSarre, B., Posto, A. L. and McKinlay, J. B. 2015. N_2 gas is an effective fertilizer for bioethanol production by *Zymomonas mobilis*. *Proc. Natl. Acad. Sci. USA.* **112**, 2222-2226.
25. Kim, I. S., Barrow, K. D. and Rogers, P. L. 2000. Nuclear magnetic resonance studies of acetic acid inhibition of rec *Zymomonas mobilis* ZM4(pZB5). *Appl. Biochem. Biotechnol.* **84-86,** 357-370.
26. Kouvelis, V. N., Davenport, K. W., Brettin, T. S., Bruce, D., Detter, C. and Han, C. S., *et al.* 2011. Genome sequence of the ethanol-producing *Zymomonas mobilis subsp. pomaceae* lectotype strain ATCC 29192. *J. Bacteriol.* **193,** 5049-5050.
27. Kouvelis, V. N., Saunders, E., Brettin, T. S., Bruce, D., Detter, C. and Han, C., *et al.* 2009. Complete genome sequence of the ethanol producer *Zymomonas mobilis* NCIMB 11163. *J. Bacteriol.* **191,** 7140-7141.
28. Kouvelis, V. N., Teshima, H., Bruce, D., Detter, C., Tapia, R. and Han, C., *et al.* 2014. Finished genome of *Zymomonas mobilis subsp. mobilis* strain CP4, an applied ethanol roducer. *Genome Announc.* **2**, e00845-13.
29. Lee, J. H., Skotnicki, M. L. and Rogers, P. L. 1982. Kinetic studies on a flocculent strain of *Zymomonas mobilis*. *Biotechnol. Lett.* **4,** 615-620.
30. Liu, C., Dong, H., Zhong, J., Ryu, D. D. and Bao, J. 2010. Sorbitol production using recombinant *Zymomonas mobilis* strain. *J. Biotechnol.* **148,** 105-112.
31. Liu, Y. F., Hsieh, C. W., Chang, Y. S. and Wung, B. S. 2017. Effect of acetic acid on ethanol production by *Zymomonas mobilis* mutant strains through continuous adaptation. *BMC Biotechnol.* **17,** 63.
32. Loos, H., Kramer, R., Sahm, H. and Sprenger, G. A. 1994. Sorbitol promotes growth of *Zymomonas mobilis* in environments with high concentrations of sugar: evidence for a physiological function of glucose-fructose oxidoreductase in osmoprotection. *J. Bacteriol.* **176,** 7688-7693.
33. Matsushita, K., Azuma, Y., Kosaka, T., Yakushi, T., Hoshida, H. and Akada, R., *et al.* 2016. Genomic analyses of thermotolerant microorganisms used for high-temperature fermentations. *Biosci. Biotechnol. Biochem.* **80,** 655-668.
34. Mohagheghi, A., Dowe, N., Schell, D., Chou, Y. C., Eddy, C. and Zhang, M. 2004. Performance of a newly developed integrant of *Zymomonas mobilis* for ethanol production on corn stover hydrolysate. *Biotechnol. Lett.* **26,** 321-325.
35. Moreau, R. A., Powell, M. J., Fett, W. F. and Whitaker, B. D. 1997. News & notes: the effect of ethanol and oxygen on the growth of *Zymomonas mobilis* and the levels of hopanoids and other membrane lipids. *Curr. Microbiol.* **35,** 124-128.
36. Pappas, K, M., Kouvelis, V. N., Saunders, E., Brettin, T. S., Bruce, D. and Detter, C., *et al.* 2011. Genome sequence of the ethanol-producing *Zymomonas mobilis subsp. mobilis* lectotype strain ATCC 10988. *J. Bacteriol.* **193,** 5051-5052.
37. Rogers, P. L., Jeon, Y. J., Lee, K. J. and Lawford, H. G. 2007. *Zymomonas mobilis* for fuel ethanol and higher value products. *Adv. Biochem. Eng. Biotechnol.* **108,** 263-288.
38. Rogers, P. L., Lee, K. J. and Tribe, D. E. 1979. Kinetics of alcohol production by *Zymomonas mobilis* at high sugar concentrations. *Biotechnol. Lett.* **1,** 165-170.
39. Seo, J. S., Chong, H., Park, H. S., Yoon, K. O., Jung, C. and Kim, J. J., *et al.* 2005. The genome sequence of the ethanologenic bacterium *Zymomonas mobilis* ZM4. *Nat. Biotechnol.* **23,** 63-68.
40. Shigeri, Y., Nishino, T., Yumoto, N. and Tokushige, M. 1991. Hopanoid biosynthesis of *Zymomonas mobili*s. *Agric. Biol. Chem.* **55,** 589-591.
41. Sootsuwan, K., Thanonkeo, P., Keeratirakha, N., Thanonkeo, S., Jaisil, P. and Yamada, M. 2013. Sorbitol required for cell growth and ethanol production by *Zymomonas mobilis* under heat, ethanol, and osmotic stresses. *Biotechnol. Biofuels.* **6,** 180.
42. Tan, F., Wu, B., Dai, L., Qin, H., Shui, Z. and Wang, J., *et al.* 2016. Using global transcription machinery engineering (gTME) to improve ethanol tolerance of *Zymomonas mobilis*. *Microb. Cell Fact.* **15,** 4.
43. Tan, F. R., Dai, L. C., Wu, B., Qin, H., Shui, Z. X. and Wang, J. L., *et al.* 2015. Improving furfural tolerance of *Zymomonas mobilis* by rewiring a sigma factor RpoD protein. *Appl. Microbiol. Biotechnol.* **99,** 5363-5371.
44. Van Uden, N. and da Cruz Duarte, H. 1981. Effects of ethanol on the temperature profile of Saccharomyces cerevisiae. *J. Basic Microbiol.* **21,** 743-750.
45. Wang, H., Cao, S., Wang, W. T., Wang, K. T. and Jia, X. 2016. Very high gravity ethanol and fatty acid production of *Zymomonas mobilis* without amino acid and vitamin. *J. Ind. Microbiol. Biotechnol.* **43,** 861-871.
46. Wang, J. L., Wu, B., Qin, H., You, Y., Liu, S. and Shui, Z. X., *et al.* 2016. Engineered *Zymomonas mobilis* for salt tolerance using EZ-Tn5-based transposon insertion mutagenesis system. *Microb. Cell Fact.* **15**, 101.
47. Wang, X., Gao, Q. and Bao, J. 2017. Enhancement of furan aldehydes conversion in *Zymomonas mobilis* by elevating dehydrogenase activity and cofactor regeneration. *Biotechnol. Biofuels.* **10,** 24.
48. Wilke, D. 1999. Chemicals from biotechnology: molecular plant genetics will challenge the chemical and the fermentation industry. *Appl. Microbiol. Biotechnol.* **52,** 135-145.
49. Xia, J., Liu, C. G., Zhao, X. Q., Xiao, Y., Xia, X. X. and Bai, F. W. 2018. Contribution of cellulose synthesis, formation of fibrils and their entanglement to the self-flocculation of

Zymomonas mobilis. *Biotechnol. Bioengin.* **115**, 2714-2525
50. Yang, S., Fei, Q., Zhang, Y., Contreras, L. M., Utturkar, S. M. and Brown, S. D., *et al.* 2016. *Zymomonas mobilis* as a model system for production of biofuels and biochemicals. *Microbial. Biotechnol.* **9,** 699-717.
51. Yang, S., Franden, M. A., Brown, S. D., Chou, Y. C., Pienkos, P. T. and Zhang, M. 2014. Insights into acetate toxicity in *Zymomonas mobilis* 8b using different substrates. *Biotechnol. Biofuels.* **7,** 140.
52. Yang, S., Land, M. L., Klingeman, D. M., Pelletier, D. A, Lu, T. Y. and Martin, S. L., *et al.* 2010. Paradigm for industrial strain improvement identifies sodium acetate tolerance loci in *Zymomonas mobilis* and *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci. USA.* **107**, 10395-10400.
53. Yang, S., Mohagheghi, A., Franden, M. A., Chou, Y. C., Chen, X. and Dowe, N., *et al.* 2016. Metabolic engineering of *Zymomonas mobilis* for 2,3-butanediol production from lignocellulosic biomass sugars. *Biotechnol. Biofuels.* **9,** 189.
54. Yang, S., Pan, C., Hurst, G. B., Dice, L., Davison, B. H. and Brown, S. D. 2014. Elucidation of *Zymomonas mobilis* physiology and stress responses by quantitative proteomics and transcriptomics. *Front. Microbiol.* **5,** 246.
55. Yang, S., Pan, C., Tschaplinski, T. J., Hurst, G. B., Engle, N. L. and Zhou, W., *et al.* 2013. Systems biology analysis of *Zymomonas mobilis* ZM4 ethanol stress responses. *PLoS One* **7**, e68886.
56. Yang, S., Pappas, K. M., Hauser, L. J., Land, M. L., Chen, G. L. and Hurst, G. B., *et al.* 2009. Improved genome annotation for *Zymomonas mobilis*. *Nat. Biotechnol.* **27**, 893-894.
57. Yang, S., Pelletier, D. A., Lu, T. Y. and Brown, S. D. 2010. The *Zymomonas mobilis* regulator *hfq* contributes to tolerance against multiple lignocellulosic pretreatment inhibitors. *BMC Microbiol.* **10,** 135.
58. Yang, S., Vera, J. M., Grass, J., Savvakis, G., Moskvin, O. V. and Yang, Y., *et al.* 2018. Complete genome sequence and the expression pattern of plasmids of the model ethanologen *Zymomonas mobilis* ZM4 and its xylose-utilizing derivatives 8b and 2032. *Biotechnol. Biofuels.* **11,** 125.
59. Yi, X., Gu, H., Gao, Q., Liu, Z. L. and Bao, J. 2015. Transcriptome analysis of *Zymomonas mobilis* ZM4 reveals mechanisms of tolerance and detoxification of phenolic aldehyde inhibitors from lignocellulose pretreatment. *Biotechnol. Biofuels.* **8,** 153.
60. Yomano, L. P., York, S. W. and Ingram, L. O. 1998. Isolation and characterization of ethanol-tolerant mutants of *Escherichia coli* KO11 for fuel ethanol production. *J. Ind. Microbiol. Biotechnol.* **20,** 132-138.
61. Zhang, K., Shao, H., Cao, Q., He, M. X., Wu, B. and Feng, H. 2015. Transcriptional analysis of adaptation to high glucose concentrations in *Zymomonas mobilis*. *Appl. Microbiol. Biotechnol.* **99,** 2009.
62. Zhang, M., Eddy, C., Deanda, K., Finkelstein, M. and Picataggio, S. 1995. Metabolic engineering of a pentose metabolism pathway in ethanologenic *Zymomonas mobilis*. *Science* **267,** 240-243.
63. Zhang, X., Wang, T., Zhou, W., Jia, X. and Wang, H. 2013. Use of a Tn5-based transposon system to create a cost-effective *Zymomonas mobilis* for ethanol production from lignocelluloses. *Microb. Cell Fact.* **12,** 41-41.
64. Zhao, N., Bai, Y., Liu, C. G., Zhao, X. Q., Xu, J. F. and Bai, F. W. 2014. Flocculating *Zymomonas mobilis* is a promising host to be engineered for fuel ethanol production from lignocellulosic biomass. *Biotechnol. J.* **9,** 362-371.
65. Zhao, N., Bai, Y., Zhao, X. Q., Yang, Z. Y. and Bai, F. W. 2012. Draft genome sequence of the flocculating *Zymomonas mobilis* strain ZM401 (ATCC 31822). *J. Bacteriol.* **194,** 7008-7009.
66. Zhao, N., Pan, Y., Liu, H. and Cheng, Z. 2016. Draft Genome Sequence of *Zymomonas mobilis* ZM481 (ATCC 31823). *Genome Announc.* **4,** e00193-16.

초록 : *Zymomonas mobilis*를 이용한 목질계 에탄올 생산을 위한 균주 개선에 관한 연구 동향

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자이모모나스 모빌리스(*Zymomonas mobilis*)는 수십 년 동안 생화학적 발효 기술을 통한 수송용 에탄올을 생산하기에 적합한 산업용 미생물로 각광을 받아왔다. 최근 이 균주의 포스트 게놈 시대 도래 및 미국 듀폰사(DuPont, USA)의 세계 최대 산업용 목질계 에탄올 생산 시설 완료 등은, 이 미생물을 이용한 산업적 에탄올 생산 공정 가시화를 위한 다양한 연구들을 파생시키고 있다. 특히, 산업용 셀룰로오스 에탄올 발효공정에 이용되는 미생물은 다양한 독성 발효 저해물질 및 물리적 스트레스에 보다 쉽게 노출 될 수 있다. 따라서 본 논문은 이 미생물이 보유한 최신 생리학적 이해와 관련 된 정보와 다양한 환경적 스트레스에 견딜 수 있는 산업적 강건성 및 산업용 균주 개발 방법에 대한 사례 및 이 균주를 이용한 가격 경쟁적인 목질계 에탄올 생산 공정 개발에 필요한 균주 개발에 대한 미래 지향적 연구 방향에 대하여 기술하였다.