**Zymomonas mobilis**: a novel platform for future biorefineries

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**Abstract**

Biosynthesis of liquid fuels and biomass-based building block chemicals from microorganisms have been regarded as a competitive alternative route to traditional. *Zymomonas mobilis* possesses a number of desirable characteristics for its special Entner-Doudoroff pathway, which makes it an ideal platform for both metabolic engineering and commercial-scale production of desirable bio-products as the same as *Escherichia coli* and *Saccharomyces cerevisiae* based on consideration of future biomass biorefinery. *Z. mobilis* has been studied extensively on both fundamental and applied level, which will provide a basis for industrial biotechnology in the future. Furthermore, metabolic engineering of *Z. mobilis* for enhancing bio-ethanol production from biomass resources has been significantly promoted by different methods (i.e. mutagenesis, adaptive laboratory evolution, specific gene knock-out, and metabolic engineering). In addition, the feasibility of representative metabolites, i.e. sorbitol, bionic acid, levan, succinic acid, isobutanol, and isobutanol produced by *Z. mobilis* and the strategies for strain improvements are also discussed or highlighted in this paper. Moreover, this review will present some guidelines for future developments in the bio-based chemical production using *Z. mobilis* as a novel industrial platform for future biofineries.

**Keywords**: *Zymomonas mobilis*, platform, biorefinery, biofuel, building block chemical

**Introduction**

There have been growing concerns about biosynthesis of fuels, desired chemicals and materials from renewable biomass resources for limited fossil resources and associated environmental issues in the past few decades [1,2]. As model industrial or laboratory organisms, *Escherichia coli* and *Saccharomyces cerevisiae* were selected as important platforms for the purpose of desired biofuels and chemicals production via metabolic engineering [3-5]. Currently, strain optimization to utilize various feedstocks (for example, starch, sugarcane, agricultural residues, industrial waste, forest residues, energy crops, et cetera) [6,7], desired products spectrum (for example, biofuels and building block chemicals), and higher yields, which have made great progress in the past decades and provided a basis for industrial applications [1-5].

As a candidate bio-ethanol producer, *Zymomonas mobilis* showed some advantages, for example, higher specific rate of sugar uptake, high ethanol yield, lower biomass production, non-requirement of controlled addition of oxygen during fermentation, et cetera [8-13]. Extensive fundamental studies on *Z. mobilis* over the last 30 years have also made this strain a promising ethanologenic organism for large-scale bio-ethanol production. On the other hand, extensive studies on different genetic techniques (including plasmid vector, expression system, transposon system, gene knockout, gene transformation, and gene function, et cetera) will help *Z. mobilis* are amenability to genetic improvement for industrial biotechnology [13]. Furthermore, strategies of strain improvement (such as conventional mutagenesis, transposon mutagenesis, adaptive laboratory evolution, and metabolic pathway engineering, et cetera), and different value-added bio-products have also been paid more and more attention in the past 20 years. Importantly, genomics and transcriptomic of *Z. mobilis* have also been developed since 2005, which will aid future metabolic engineering and synthetic biology in strain improvement for industrial...
applications [14]. Selected milestones in \textit{Z. mobilis} research are summarized in Figure 1. Currently, three subspecies (subsp.) of \textit{Z. mobilis} have been found, including \textit{Z. mobilis} subsp. \textit{mobilis}, \textit{Z. mobilis} subsp. \textit{pomaceae} and \textit{Z. mobilis} subsp. \textit{Francensis} [15-19]. All strains have also been summarized in the Ph D thesis of So Lok-yan (University of Hong Kong) and other review articles [19]. Among these strains, ATCC 31821 (ZM4), ATCC 10988 (ZM1), ATCC29191 (ZM6), CP4, and NCIMB 11163 from \textit{Z. mobilis} subsp. \textit{mobilis}, ATCC 29192 from \textit{Z. mobilis} subsp. \textit{pomaceae}, which were well-characterized by previous studies on the level of physiology, biochemical, fermentation, genetics, metabolism, and omics. These strains are regarded as a model organism in \textit{Z. mobilis} research or industrial applications.

In general, \textit{Z. mobilis} may play a critical role as a novel platform in industrial biotechnology for the development of a green replacement for petrochemical products. In this paper, we review some critical research progress on \textit{Z. mobilis} for its use as a platform for the production of ethanol and other buck chemicals from biomass.

\textbf{Review}

\textbf{Genetic background of \textit{Z. mobilis}}

Currently, general genetic tools have been developed in \textit{Z. mobilis} since the 1980s, including native plasmids, broad host-range vectors or shuttle vectors, expression system, gene transfer, promoter, and reporter gene, as reviewed in other articles [8,11,13]. Specific gene knockout, genomics, and transcriptomics will be emphasised as below.

\textbf{Specific gene knockout}

The development of gene deletion approaches have been performed for gene function and there has also been greatly improved metabolic engineering of \textit{Z. mobilis}. Currently, different methods, including insertional mutant, suicide plasmid-based mutant construction, site-specific FLP recombinase, fusion-PCR-based construction technique, and transposon mutagenesis, have been employed for inactivating specific genes of \textit{Z. mobilis}. Up to date, many genes, such as pyruvate decarboxylase (\textit{pdc}, ZMO1360), alcohol dehydrogenase (\textit{adhB}, ZMO1596), lactate dehydrogenase (\textit{ldhA}, ZMO1237), NADH dehydrogenase (\textit{ndh}, ZMO1113), RNA-binding protein \textit{Hfq} (\textit{hfq}, ZMO0347), hydroxylamine reductase (\textit{nhaA}, ZMO0117), glucose-fructose oxidoreductase (\textit{gfo}, ZMO0689), aldo/keto reductase (\textit{himA}, ZMO0976), restriction-modification (R-M) systems-related gene (ZMO0028, ZMO1933, ZMO1934, ZMO1934, ZMO0575), cytochrome-related gene (\textit{cytC}, \textit{cytB}, \textit{ctbD}, ZMO0957, ZMO1572) et cetera, which were selected as targets for improvement of some specific phenotype (summarized in Table 1).

\textbf{Sequenced genome of different \textit{Z. mobilis} strains}

Genome sequencing technology provides opportunities for fundamental insights and facilitates strain development [35]. Seo \textit{et al.} reported the first genome sequence of \textit{Z. mobilis} ZM4 in 2005. The complete genome of \textit{Z. mobilis} ZM4 contains a 2,056,416-bp circular chromosome and five circular plasmids [9]. The complete genome sequence of other \textit{Z. mobilis} strains have also been reported since 2005 [36-41]. All strains contain a circular chromosome and types of plasmids. However, genome sizes are various among these strains, ranging from 2.01 to 2.22, with two to six plasmids existing (Table 2). Although the genome of seven strains has been sequenced by different organizations, the comparative genome analysis has not been reported in public.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{Figure1.png}
\caption{Selected milestones in \textit{Z. mobilis} research.}
\end{figure}
Transcriptome or gene expression of Z. mobilis

With different genome projects of Z. mobilis performed, further comparative genomics or global expression analysis could provide some guidelines for strain improvement in the future. Currently, many researchers are also focusing on transcriptomic profiling of Z. mobilis to better understand the network of gene or metabolic regulation. Especially, DNA microarray techniques or DNA sequencing have been used to identify differential gene expression under nutrition limitation, environmental stress (that is, heat stress, ethanol, furfural, et cetera). To date, there are ten datasets (including some unpublished data) from Gene Expression Omnibus (GEO) database (Table 2). For example, transcriptomic profiling of ZM4 during aerobic and anaerobic fermentations have been investigated for the first time [42]. Transcriptomic profiling of ZM4 in response to ethanol and furfural stress were also performed by our laboratory [43,44]. Integrated “omics” approach (that is transcriptomic, proteomic and metabolic) was also used for studying the molecular mechanisms of ethanol stress response in ZM4 for the first time [45]. Expression data for ZM4 growing in rich and minimal media,

### Table 1 Summary of specific gene knockout in Z. mobilis

| Gene inactive | Method | Description | References |
|---------------|--------|-------------|------------|
| Extracellular sucrase gene (sacC, ZMO0375) | Insertional mutant | Improves levan production | [20] |
| Restriction-Modification (R-M) systems related gene (ZMO0028, ZMO1932, ZMO1933, ZMO1934, ZMO1935) | Insertional mutant or Homologous recombination | Increased transformation efficiency | [21,22] |
| pdc (ZMO1360) | Homologous recombination | Lower ethanol and lactate yield, and higher succinate concentration from glucose | [23] |
| adhB (ZMO1596) | Transposon mutagenesis | Reduced himA activity and increased ethanol production compared to parental strains when cultured in a mixed-sugar medium containing xylose, especially in the presence of acetate | [24,25] |
| ndh (ZMO1113) | Insertional mutant | Low respiration rate, higher cell growth and ethanol yield under aerobic conditions | [26,27] |
| hflq (ZMO0347) | pKNOCK suicide plasmid-based mutant construction | More sensitive to multiple lignocellulosic pretreatment inhibitors and has an increased lag phase duration and/or slower growth depending upon the conditions; and verified that hflq plays a role in tolerance to multiple biomass pretreatment inhibitors, including acetate, vanillin, furfural, and HMF | [28] |
| nhaA (ZMO0117) | Insertional mutant | Cell growth decreased under sodium acetate condition | [29] |
| Xylose reductase (XR, ZMO0976) | Homologous recombination | Improvement of xylose utilization | [30] |
| gfo (ZMO0689) | Site-specific FLP recombinase | Improves growth and ethanol production without formation of sorbitol as a by-product in sucrose medium, but yields opposite effects in high glucose | [31] |
| gfo (ZMO0689) | Homologous recombination (fusion-PCR-based construction technique) | Reduction of cell growth and ethanol production under osmotic, heat and ethanol stresses | [32] |
| cytC | Insertional mutant | Exhibits filamentous shapes and reduction in growth under a shaking condition at a high temperature | [33] |
| cytB (ZMO0957), ctdB (ZMO1572) | Insertional mutant | Low respiration capacity when cultivated anaerobically | [34] |
| psp operon (ZMO1061-ZMO1065) | Homologous recombination (fusion-PCR-based construction technique) | Multiple phenotypes | Our laboratory, unpublished data |
| Mutant library | Transposon mutagenesis | Multiple phenotypes | Our laboratory, unpublished data |
heat-shocked, or at high ethanol were also performed by Lawrence Berkeley Laboratory (unpublished data). Genome changes associated with \textit{Z. mobilis} sodium acetate-tolerant mutant (AcR) was also reported by Yang et al. In this study, next-generation sequencing (NGS), comparative genomics, transcriptomics, and genetics were used to elucidate the molecular mechanism of AcR sodium acetate tolerance. Especially, a key gene, \textit{nhaA} (ZMO0119), which conferred sodium acetate (NaAc) tolerance in \textit{Z. mobilis} [29]. ZM401 (a flocculent mutant strain of \textit{Z. mobilis}) was also studied by using genome-wide transcriptomic technology, which provided a deep understanding for evidence related to phenotypic changes associated with its cell-cell attachment behavior. These expression data indicate that cellulose and synthesis flagella-related proteins synthesis play an important role in its special flocculent behavior in ZM401 [46]. These studies will provide insights into molecular response to environmental stress in \textit{Z. mobilis} or help to construct more resistant strains for ethanol or other chemical production in the future. In conclusion, those transcriptomic profiling generated in these studies will likely serve as useful reference data for industrial strain development at the level of systems biology in the future.

### Strain improvement for \textit{Z. mobilis}

#### Strain improvement by conventional mutagenesis

Traditionally, strain improvement was achieved mainly by mutagenesis and selection, which are still very useful in \textit{Z. mobilis}. Currently, different mutagenesis agents, including UV light, 1-methyl-3-nitro-1-nitrosoguanidine (MNNG), and 1,3-propanesultone (PS) have been used. However, these methods may not be effective for strain improvement in \textit{Z. mobilis}, especially for the flocculent strain ZM401. Therefore, researchers have developed alternative methods to improve strain properties, such as evolutionary engineering and adaptive laboratory evolution (ALE).

### Table 2 Genomics, transcriptome or gene expression in different \textit{Z. mobilis} strains

| Sequenced genomea | Accession number | Description | Size (Mb) | Plasmids | Protein | References |
|-------------------|-----------------|-------------|-----------|----------|---------|------------|
| \textit{Z. mobilis} ZM4 (ATCC31821) | NC_006526.2 | Transcriptomic profiling of ZM4 during aerobic and anaerobic fermentations | 2.06 | 5 | 1,738 | [9] |
| NCIMB11163 | NC_013355.1 | Expression profiling of ZM4 in response to furfural stress | 2.22 | 3 | 1,884 | [36] |
| ATCC 29191 | NC_018145.1 | Transcriptomic profiling of ZM4 in response to ethanol stress | 2.01 | 3 | 1,709 | [37] |
| ATCC 29192 | NC_015709.1 | Systems biology analysis of ZM4 ethanol stress responses | 2.06 | 2 | 1,748 | [38] |
| ATCC 10988 | NC_017262.1 | Comparison of gene expression and mutant fitness in ZM4 | 2.14 | 6 | 1,803 | [39] |
| ZM401 (ATCC 31822) | Draft genome sequence | Expression data for ZM4 growing in rich and minimal media, heat-shocked, or at high ethanol | 2.04 | Not found | 1,910 | [40] |
| CP4 (NRRL B-14023) | NC_022900.1 (CP006818.1) | Genome changes associated with \textit{Z. mobilis} sodium acetate-tolerant mutant (AcR) | 2.16 | 5 | 1,840 | [41] |

#### Transcriptome or gene expression

| \textit{Z. mobilis} strain | Accession number | Description | References |
|---------------------------|-----------------|-------------|------------|
| ZM4 (ATCC31821) | GSE10302 | Transcriptomic profiling of ZM4 during aerobic and anaerobic fermentations | [42] |
| GSE37848 | Expression profiling of ZM4 in response to furfural stress | [43] |
| GSE39558 | Transcriptomic profiling of ZM4 in response to ethanol stress | [44] |
| GSE21165 | Systems biology analysis of ZM4 ethanol stress responses | [45] |
| GSE39466 | Comparison of gene expression and mutant fitness in ZM4 | Lawrence Berkeley Laboratory, unpublished data |
| GSE51870 | Expression data for ZM4 growing in rich and minimal media, heat-shocked, or at high ethanol | Lawrence Berkeley Laboratory, unpublished data |
| ZM4 (AcR) | GSE18106 | Genome changes associated with \textit{Z. mobilis} sodium acetate-tolerant mutant (AcR) | [29] |
| RDM-4 strain of \textit{Z. mobilis} | GSE22355 | Expression analysis of a respiration-deficient mutant of \textit{Z. mobilis} ZM6 | Faculty of Food and Nutrition, Beppu university |
| ZM401 | Not deposited | Genome-wide transcriptomic analysis of a flocculent strain of \textit{Z. mobilis} ZM401 | [46] |
| ZM4 (ATCC31821) | GSE49620 | Transcriptional responses of \textit{Z. mobilis} to osmotic shock of high glucose concentration | Unpublished data, performed by Sichuan University and Biogas Institute of Ministry of Agriculture |

*aDetailed information on genome projects of \textit{Z. mobilis} can be accessed at the NCBI Microbial Genomes Resources database: http://www.ncbi.nlm.nih.gov.genome/?term=zymomonas+mobilis or the Genomes OnLine Database at: http://www.genomesonline.org/.*
(NTG), caffeine, ethyl methane sulfonate (EMS), et cetera, were used for \textit{Z. mobilis} phenotype improvement. Many mutants were obtained by these mutageneses, that is, auxotrophic, ethanol and salt-tolerant, acetalddehyde-tolerant, osmotolerant, thermostolerant, sucrose-hypertolerant, acid-tolerant, fructose-negative, glucose-negative, mannitol-utilizing, levan-producing, and antibiotic-sensitive strains, et cetera (as reviewed by other authors) [8]. Among these mutants, environmental stress-tolerant mutant, and antibiotic-sensitive strains have showed some potential in industrial applications. For example, the acetate-tolerant \textit{Z. mobilis} mutant (AcR) was generated by chemical mutagenesis and selection in the presence of acetate [47], and used as a host for constructing of engineered tolerant \textit{Z. mobilis} strain for bio-ethanol production, that is ZM4/AcR (pZB5) [48-50].

\textbf{Strain improvement by transposon mutagenesis}

Transposon mutagenesis has also provided an alternate mutational approach in \textit{Z. mobilis}. Although different transposons, including \textit{Tn5} and \textit{Tn10} [51], \textit{Tn951} [52] and \textit{Tn1725} [53], which are carried by broad host-range plasmids, have been successfully transferred into \textit{Z. mobilis}, no transposition event have been found. Moreover, Carey et al. first found that plasmid pGC91.14 (RPl::Tn951) was stable in \textit{Z. mobilis} at 30°C, and the lac operon encoded by Tn 951 was expressed successfully in \textit{Z. mobilis} [52]. Pappas et al. also compared of the stability of different transposable elements \textit{Tn5}, \textit{Tn50I} or mini Mu in \textit{Z. mobilis}, and the plasmid pULB113 (RPl::mini Mu) exhibited higher stability than others. With the help of mini Mu transposon, a large number of independent and stable auxotrophic mutants with polyauxotrophs, cysteine, methionine and isoleucine requiring-isolates were obtained [54]. The study proved that transposon mutagenesis is an extremely powerful tool for mutant construction in \textit{Z. mobilis} [54,55]. For example, \textit{Tn5} transposon was also used for construction of recombinant strain for ethanol production [56]. Actually, there are some transposon elements in \textit{Z. mobilis} strains. For example, IS5-like insertion sequence, designated \textit{ISZm1068}, was firstly isolated from \textit{Z. mobilis} CP4, which was kept active in \textit{E. coli} and led to plasmid replicon fusions [57].

\textbf{Strain improvement by adaptive laboratory evolution (ALE)}

Adaptive laboratory evolution (ALE) has emerged as a valuable method in metabolic engineering for strain development and optimization [58-62], and has been used successfully in model organisms such as \textit{E. coli} [63,64] and \textit{S. cerevisiae} [65-68]. Previous studies demonstrated that adaptation and metabolic engineering can be used synergistically for strain improvement. Recently, ALE strategy was also employed for \textit{Z. mobilis} strain improvement. For example, an adaptive mutation procedure was developed for screening of acetic acid-tolerant \textit{Z. mobilis}, and many adapted mutants obtained for further use in bio-ethanol production [69]. Agrawal et al. also used this method to select a highly efficient xylose-fermenting \textit{Z. mobilis} strain A3 [70]. These two studies demonstrated that the ALE method might be used as a powerful metabolic engineering strategy for improving certain features of \textit{Z. mobilis} in the future, for example, inhibitor tolerance or substrate utilization.

\textbf{Increase in the substrate utilization range of \textit{Z. mobilis}}

Extensive studies or reviews on ethanol production from sugarcane, molasses, starch, and glucose by \textit{Z. mobilis} have been performed by many authors [8,10-13,19,71,72]. Based on the consideration of some debates about food security [73], environmental degradation [74] and other issues, developing lignocellulosic feedstocks to substitute corn or sugarcane for bioenergy production will be an inevitable trend in the future [75]. Currently, recombinant \textit{Z. mobilis} capable of simultaneous fermentation of pentose and hexose sugars from lignocellulosic hydrolysates to ethanol have been achieved since 1995. The brief research history is shown in Figure 2.

In 1995, Zhang et al. from the National Renewable Energy Laboratory (NREL) constructed a recombinant \textit{Z. mobilis} CP4 (pZB5) strain by introducing two operons encoding xylose assimilation and pentose phosphate pathway enzymes from \textit{E. coli} into \textit{Z. mobilis} for the first time, which could ferment pentose sugar and allowing for growth on xylose with 86% ethanol yield [76]. Based on Zhang’s research, another arabinose-fermenting recombinant \textit{Z. mobilis} CP4 (pZB206) strain was also constructed by introducing five arabinose metabolism-related genes from \textit{E. coli} into \textit{Z. mobilis} CP4 in 1996, which could ferment arabinose sugar and produced ethanol at 98% of theoretical yield [77]. For co-fermenting glucose, xylose, and arabinose to ethanol simultaneously, co-culture processes of \textit{Z. mobilis} ATCC 39676 (pZB4L) and ATCC 39676 (pZB206) have been performed, which showed 72.5% of theoretical ethanol yield [78]. However, both xylose-fermenting strain and xylose had a significant effect on the performance of the arabinose utilization strain. Based on these considerations, Zhang et al. constructed a single \textit{Z. mobilis} 206C (pZB301) in 1998, which could ferment mixture sugars to ethanol via 82 to 84% theoretical yield [79]. However, all recombinant strains were constructed by antibiotic-resistant plasmid; addition of antibiotics to maintain stability for large-scale fermentations is highly undesirable. For enhancing its genetic stability, all seven genes necessary for pentose utilization were integrated into the\textit{Zymomonas} genome and a stable \textit{Z. mobilis} AX101 strain obtained in 2002, which could ferment a hextose and pentose mixture via a preferential order [80].
Although a strain capable of co-fermentation of all three sugars was achieved, all recombinant strains were sensitive to acetic acid stress. For example, nuclear magnetic resonance (NMR) studies found that acetic acid could inhibit efficiency of xylose utilization in _Z. mobilis_ ZM4 (pZB5) [81]. Different strategies were developed to improve the tolerance of acetic acid and xylose utilization. For example, Lawford and Rousseau et al. developed a process via addition of extra glucose in acetic acid-containing media for improving fermentation performance of recombinant _Zymomonas_ [82]. Recombinant plasmid pZB5 was also transferred into an acetic acid-tolerant strain (ZM4/AcR) [47], and a mutant recombinant _Z. mobilis_ ZM4/AcR (pZB5) strain with increased acetate resistance was obtained [48]. Overexpression of xylulokinase in a xylose-metabolising recombinant strain was also performed, and resulted in another recombinant ZM4/AcR (pZB5) strain with increased acetate resistance was obtained [48]. Overexpression of xylulokinase in a xylose-metabolising recombinant strain was also performed, and resulted in another recombinant ZM4/AcR (pZB5) strain with increased acetate resistance was obtained [48]. Overexpression of xylulokinase in a xylose-metabolising recombinant strain was also performed, and resulted in another recombinant ZM4/AcR (pZB5) strain with increased acetate resistance was obtained [48]. Overexpression of xylulokinase in a xylose-metabolising recombinant strain was also performed, and resulted in another recombinant ZM4/AcR (pZB5) strain with increased acetate resistance was obtained [48]. Overexpression of xylulokinase in a xylose-metabolising recombinant strain was also performed, and resulted in another recombinant ZM4/AcR (pZB5) strain with increased acetate resistance was obtained [48]. Overexpression of xylulokinase in a xylose-metabolising recombinant strain was also performed, and resulted in another recombinant ZM4/AcR (pZB5) strain with increased acetate resistance was obtained [48]. Overexpression of xylulokinase in a xylose-metabolising recombinant strain was also performed, and resulted in another recombinant ZM4/AcR (pZB5) strain with increased acetate resistance was obtained [48]. Overexpression of xylulokinase in a xylose-metabolising recombinant strain was also performed, and resulted in another recombinant ZM4/AcR (pZB5) strain with increased acetate resistance was obtained [48]. Overexpression of xylulokinase in a xylose-metabolising recombinant strain was also performed, and resulted in another recombinant ZM4/AcR (pZB5) strain with increased acetate resistance was obtained [48]. Overexpression of xylulokinase in a xylose-metabolising recombinant strain was also performed, and resulted in another recombinant ZM4/AcR (pZB5) strain with increased acetate resistance was obtained [48].

In 2013, a cost-effective recombinant _Z. mobilis_ HYMX was constructed by integrating seven genes (Pfu-sHSP, yfdZ, metB, xylA, xylB, tktA and talB) into the genome of _Z. mobilis_ CP4 via Tn5 transposon, which showed tolerance to multiple stresses, high yield and stable genetic characteristics [56].

Furthermore, fermentation characteristics of different recombinant strains were also analyzed in the past decade [30,49,56,78-81,83,84,86-89]. Importantly, fermentation performance of three best recombinant strains form different platforms used for cellulosic ethanol production, _E. coli_ KO11, _S. cerevisiae_ 424A (LNH-ST) and _Z. mobilis_ AX101, which were compared with cellulosic material for the first time. Especially, _Z. mobilis_ AX101 showed the highest rate of glucose consumption and lowest yield of byproducts [88]. These results also indicate that the metabolic pathway of _E. coli_ KO11 and _Z. mobilis_ AX101 are more effective in fermenting ethanol from the related yeast pathway of the consumed sugars [88]. However, utilization of xylose in lignocellulosic hydrolysate and growth robustness of recombinant _Z. mobilisare_ also required to improve in the future. Moreover, different lignocellulosic feedstocks, such as agro-industrial wastes [90], sugarcane bagasse [91], oat hull [92], corn stover [49,93], bamboo residues [94], and various hydrolysates produced by Arkenol Technology [50], have also been used for ethanol production by _Z. mobilis_. In general, these studies will provide a deep basis for the ethanol industry in the future.
Although different engineered Z. mobilis strains have also been successfully constructed by introducing desirable genes as previously mentioned, conversion of cellulosic biomass into ethanol directly is also a considerable task for ethanol production. Recently, there has been development of consolidated bioprocessing (CBP) - a combination of cellulase production, cellulose hydrolysis and fermentation into a single step, which is regarded as an alternative approach with outstanding potential [95,96]. In 2010, two cellulolytic enzymes, E1 and GH12 from Acidothermus cellulolyticus were successfully expressed in Z. mobilis via a native secretion signal peptide [97]. Five cellulolytic enzymes from bacteria isolated from the gut of phytophagous insects were also transferred into Z. mobilis, and all the resulting recombinants fermented pretreated cellulosic feedstocks directly into ethanol [98]. In another study, six genes encoding cellulolytic enzymes (CenA, CenB, CenD, CbhA, CbhB, and Cex) from Cellulomonas fimi and other cellulolytic enzymes (cenA, bgl) from Ruminococcus albus were also introduced and co-expressed successfully in Zymobacter palmae, which enabled Z. palmae to efficiently ferment a water-soluble cellulosic polysaccharide to ethanol [99]. Although the recombinant Z. mobilis strains need to be improved further by simultaneous expression of additional cellulase genes, all these results also indicate that Z. mobilis could be serving as an important CBP platform organism.

Other value-added bio-products production by Z. mobilis Sorbitol and bionic acid production

In 2013, the US Department of Energy (DOE) published 12 topvalue-added building-block chemicals from biomass [100]. Representative chemicals, including four carbon 1,4-diacids (sucinic, fumaric, and malic), 2,5-furan dicarboxylic acid (FDCA), 3-Hydroxypropionic acid (3-HPA), aspartic acid, glutamic acid, glucaric acid, itaconic acid, levulinic acid, 3-Hydroxybutyrolactone, glyceral, sorbitol, xylitol/arabinitol. Sorbitol was identified as one of the top 12 building block chemicals by the US DOE [100], and could be produced by Z. mobilis.

Actually, Barrow et al. found a phenomenon that ethanol yield was decreased when Z. mobilis grown on sucrose or mixtures of glucose plus fructose medium. Further study by NMR spectroscopy indicated that the reason for reduced ethanol yield was due to sorbitol formation from fructose [101]. Leigh et al. identified a proposed metabolic pathway for the production of sorbitol in Z. mobilis [102]. Zachariou and Scopes et al. demonstrated glucose-fructose oxidoreductase (GFOR) and glucono-\(\alpha\)-glucosone (GL) are responsible for sorbitol production, and gluconate intermediate could be converted to ethanol via the Entner-Doudoroff (ED) pathway [103]. These extensive studies demonstrated that Z. mobilis could produce sorbitol in a one-step reaction via GFOR, which is so far only known from this bacterium.

Based on these studies, many researchers developed different processes for producing sorbitol or gluconic acid production by Z. mobilis via whole cells, permeabilized cells or immobilized cells (as shown in Table 3). For example, Chun and Rogers et al. developed a simultaneous process for sorbitol and gluconic acid, 290 g/L of sorbitol and 283 g/L of gluconic acid were yielded from 60% total sugar solution (300 g L\(^{-1}\) glucose and 300 g L\(^{-1}\) fructose) after a 15-h reaction with Z. mobilis-permeabilized cells [104]. Rehr et al. found no gluconic acid formation when using glucose-grown cells for the conversion of equimolar fructose and glucose mixtures. However, nearly 295 g L\(^{-1}\) of sorbitol and gluconic acid were produced using cetyltrimethylammonium bromide (CTAB)-treated cells [105]. These results supported that gluconate intermediate converted to ethanol via the ED pathway [103,106]. Silveira et al. found that the yield of sorbitol and gluconic acid increased with substrate concentration [107]. Cazetta et al. investigated sorbitol production from sugar cane molasses by Z. mobilis, which showed the best conditions for sorbitol production containing 300 g/L total reducing sugars (TRS) in the culture medium [108]. Actually, to improve the sorbitol yield, various cell permeabilization methods, that is toluene [104], dried Z. mobilis cells, CTAB [105], metal ions [109], which inhibited key enzymes of the ED pathway and led to decreased ethanol concentration.

Although Lactobacillus casei [110] and Lactobacillus plantarum [111] were also engineered for sorbitol production, sorbitol with a yield up to 0.65 to 0.67 mol/mol glucose [111,112], the conversion rate of sugar and yield of sorbitol are lower when compared to Z. mobilis. So, Z. mobilis showed some advantages of sorbitol production, including a one-step reaction via GFOR, higher conversion rate of sugar and yield, and higher value of byproduct. It may be used for sorbitol production in an industrial scale in the future.

| Substrate | Biocatalyst | Products (g/L) |
|-----------|-------------|----------------|
| 600       | Permeabilized cell | 290 283 [104] |
| 100       | Whole cell     | 240 ND [105]  |
| 300       | Permeabilized cell | 295 295 [107] |
| 650       | Whole cell     | 12 15 [107]   |
| 100       | Whole cell     | 105 50 [107]  |

Table 3 High yield of sorbitol and gluconic acid production by Z. mobilis
However, activity of GFOR in wild-type *Z. mobilis* is very low and regulated by glucose concentration [103]. For further improvement of sorbitol production, overexpression of GFOR is an attractive strategy to improve its efficiency. As reported by Liu *et al.*, an engineered strain harboring plasmid pHW20a-gfor showed higher sorbitol yield than the wild strain [109]. On the other hand, although *Z. mobilis* could convert a mixture of glucose and fructose into sorbitol with high efficiency, the cost of the substrate may be very high. No natural feedstocks could meet the demand of high sugar-concentration. So, further research need to be carried out for searching for cheaper feedstocks or into the development of a novel process for conversion of lower sugar-concentration. Fortunately, the metabolic pathway of sorbitol and gluconic acid are clear [103,113], and gene regulation of gfor has also been studied by many research groups [32,114]. Loos *et al.* described a sorbitol-related protection mechanism of osmotic stress in concentrated sugar media [114]. Further research also indicates that sorbitol is required for cell growth and ethanol production under heat, ethanol, and osmotic stresses in *Z. mobilis* [32]. These clues will provide a chance for improving sorbitol and gluconic acid yield through metabolic engineering.

Furthermore, for determination of the substrate spectrum of GFOR, Satory *et al.* first reported that GFOR enzyme from *Z. mobilis* can oxidize different aldose sugars into corresponding aldonic acid when D-Fructose is used as the corresponding acceptor substrate. The conversion efficiency ranges from 9 to 84%, which shows a broad spectrum of substrates for the enzyme [115]. The study indicated that GFOR could be potentially used for other bionic acid production, that is, lactobionic acid (LBA), a lactose derivative that has many value-added applications in cosmetics, pharmaceutical or biomedicines, food, and chemical industries, as reviewed by Alonso *et al.* [113]. Lactose oxidation by GFOR was also performed by Satory *et al.*, which showed a high productivity of 110 g/L·d⁻¹ in a continuously stirred tank reactor (CSTR) after operating for 70 h [115]. Bioconversion of a mixture of fructose and lactose into sorbitol and LBA with immobilized cells of *Z. mobilis* in calcium-alginate has also been reported [116,117]. Other bionic acids, such as maltobionic, xylonic, galactonic acid, arabinonic acid, mannonic acid and cellobionic acid, should also be performed in the future, which shows another important application for *Z. mobilis* (as shown in Figure 3).

**Levan production by *Z. mobilis***

Levan is a fructose polymer with potential importance in food technology or medical applications [118]. Actually, Dawes and Ribbons *et al.* first found that reduction of ethanol yield has been attributed to levan formation when *Z. mobilis* grown on sucrose medium [119]. Further research also verified that ethanol-yield reduction might be due to sorbitol and levan formation [101,102,120]. For example, Beker *et al.* developed a simultaneous sucrose bioconversion into ethanol and levan by *Z. mobilis*, and

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**Figure 3** Reaction scheme for the production of bionic acid and sorbitol via glucose-fructose oxidoreductase (GFOR) and glucono-Δ-lactonase (GL) of *Z. mobilis*. 
the levan yield of 0.22 g/g and the productivity of 3.2 g/L/h obtained [121]. Yoshida et al. and other researchers also found Z. mobilis could produce a high yield of levan when cultivated in sucrose medium [122-124]. Calazans et al. also found that levans produced by Z. mobilis strains have anti-tumor activities, and its molecular weight was also determined [125,126]. Previous studies verified that intracellular sucrase (SacA), extracellular levansucrase (SacB) and extracellular sucrase (SacC) contribute to sucrase hydrolysis in Z. mobilis [127]. Based on its genetic and biochemical studies, Senthilkumar et al. constructed a SacC mutant via the insertional mutant method, and higher yield of levan was obtained [20]. To avoid unnecessary supplementation with vitamins and mineral salts, low-cost effective substrate needs be used for levan production in Z. mobilis [128]. Levan production in batch and continuous fermentation systems by Z. mobilis B-14023 was also investigated recently [129]. These extensive studies indicate that Z. mobilis may be used for industrial levan production for some purposes.

Succinic acid production by Z. mobilis

Succinic acid was identified as one of the top 12 building-block chemicals by the US DOE[100]. Transparency Market Research also published a new report, Succinic Acid Market - Global Industry Analysis, Size, Share, Growth, Trends and Forecast, 2012-2018, in October 2013, which predicted that its market will be expected to reach USD 836.2 million by 2018. Based on these considerations, biological production of succinic acid from abundant and available biomass has become a topic of worldwide interest. Currently, different natural succinate-producing or genetically modified strains, such as Actinobacillus succinogenes, Anaerobiospirillum succiniproducens, Mannheimia succiniciproducens, Bacteroides fragilis, and Corynebacterium sp. have been used for bio-based succinic acid production from different feedstocks [130,131]. Other strains, including E. coli [132,133], and S. cerevisiae [134,135] have also been engineered for succinic acid production. Although these strains showed some advantages for succinic acid production, the process of fermentation is anaerobic and kinds of byproducts are formed. Recently, Lee et al. constructed a genome-scale metabolic model of Z. mobilis (ZmoMBEL601), which suggested a possible strategy for succinic acid production by disrupting pyruvate decarboxylase (pdc, ZMO1360) or alcohol dehydrogenase (adhlB, ZMO1596) and D-lactate dehydrogenase (ldhA, ZMO1237) simultaneously [136]. Although this conclusion is based on the metabolic model, the higher yield of succinic acid will likely achieved in the future. Actually, Seo et al. have constructed an engineered Z. mobilis for succinic acid production by redirecting metabolic pathways upon gene knockout of pdc and ldhA. The double gene-knockout strain ZM4 (pdc− ldhA) has produced 1.46 mol succinate from 1 mol glucose, which showed 95% theoretical yield, and agrees well with the metabolic model ZmoMBEL601 [23]. Based on these studies, a suggested pathway for succinic acid may be proposed, as shown in Figure 4.

Other studies in silico or stoichiometric analysis of the central metabolism of Z. mobilis are valuable, for instance, Widiastuti et al. have also confirmed the functional role of pdc and adhl genes during ethanol production in Z. mobilis via a genome-scale metabolic network (izm363) [137]. A medium-scale model based on stoichiometric analysis of central metabolism was also performed by Penttjus et al. [138]. These studies will also help us to gain a deep understanding of its special physiological characteristics or re-direct its metabolic pathway for production of target products in the future.

Isobutanol production

Isobutanol has also been paid more and more attention in recent years for its advantages over bio-ethanol as a liquid fuel [2,139]. Engineered strains for isobutanol production in E. coli [139-141], S. cerevisiae [142-144], Corynebacterium glutamicum [143,145-147], Bacillus subtilis [148], and fungal-bacterial consortia [149], have been engineered or reviewed in previous studies. Recently, an engineered Z. mobilis strain was also constructed for isobutanol production via metabolic pathway engineering: 2-ketoisovalerate decarboxylase (kivd) gene and alcohol dehydrogenase (adhl) gene from Lactococcus lactis were introduced into Z. mobilis ZM4, which led to isobutanol accumulation. Although the yield of isobutanol is very low, an engineered Z. mobilis is first used for producing the isobutanol. Higher yield may be obtained by disruption of key genes of the ED pathway (as shown in Figure 2) or addition of the extra biosynthesis pathway of alanine (for example, alaD, L-alanine dehydrogenase). Actually, alaD gene Bacillus sphaericus was also cloned and introduced into Z. mobilis, and 7.5 g/L alanine was excreted in the recombinant strain [150].

Other products

Isoprenoids represent another wide group of chemically active compounds, which could be produced by engineered microorganisms, and show a broad range of applications [151,152]. Actually, Z. mobilis has the highest total hopanoid content (30 mg/g DCW, dry cell weight) among all bacteria, which leads to more tolerance by increasing the hopanoid content [153,154]. Further research has also verified its biosynthesis pathway via the methylyerythritol phosphate (MEP) pathway [155]. Moreover, biosynthesis pathway of hopanoid lipids and its regulation have also been characterized on the genetic level, which formed a biosynthetic operon [43,44,156-158]. These results indicated that Z. mobilis has higher activity of the isoprenoid biosynthesis pathway, which may be
potentially used for isoprenoid compounds production and reflects a novel application for \textit{Z. mobilis}. Actually, a group of plasmid-encoded carotene biosynthetic genes (\textit{crtB, crtE, crtI, crtY}) have been introduced into \textit{Z. mobilis} via conjugation, resulting in production of $\beta$-carotene \cite{159}. Several genes from the thermally dimorphic fungus \textit{Penicillium marneffei} with predicted terpene synthase function were also selected for functional analysis and evaluation of their potential for the bioproduction of isoprenoid compounds in \textit{Z. mobilis} (as shown in the PhD thesis of So Lok-yen, University of Hong Kong). Although these studies represent preliminary work, with deeper understanding of its biosynthesis pathway, \textit{Z. mobilis} shows great potential for isoprenoid compounds production in the future.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure4.png}
\caption{Metabolic pathways for the production of the high-value products by using \textit{Z. mobilis} as platform. The solid lines indicate \textit{Z. mobilis} native pathways and the dotted lines refer to the recombinant pathway obtained by metabolic engineering strategies. gfr, glucose-fructose oxidoreductase; ldhA, lactate dehydrogenase; pdc, pyruvate decarboxylase; gnl, glucono-\(\delta\)-lactone; adc, acetoacetate dehydrogenase; adhE, secondary alcohol dehydrogenase; adhF, alcohol dehydrogenase; adhE2, secondary alcohol dehydrogenase; atoAD, acetyl-CoA:acetoacetyl-CoA transferase; atoB, acetyl-CoA acetyltransferase; bcd, butyryl-CoA dehydrogenase; crt, crotonase; ctfAB, \(\delta\)-hydroxybutyryl-CoA dehydrogenase; fad, fatty acid dehydrogenase; thl, thiolase; kivd, ketoisovalerate dehydrogenase.}
\end{figure}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure5.png}
\caption{General process of fuel or chemical production by \textit{Z. mobilis}.}
\end{figure}
Conclusions
Based on the previous and our reviews, Z. mobilis is firstly being developed as an effective ethanologenic by engineering strain improvement, including utilization of xylose and arabinose in addition to glucose. Undoubtedly, Z. mobilis has shown desirable characteristics for its special metabolic pathway. The scientific and technological progress of Z. mobilis have also made a significant contribution to the bioethanol industry. Compared with E. coli, Z. mobilis has high restriction-modification enzyme activity, and cannot be contaminated by bacteriophages [13]. It is fairly osmo-tolerant and can hence tolerate very high sugar concentrations, which is an advantage in fermentation in a high-sugar medium. Its smaller genome and simple metabolic pathway, also lead to less byproducts formation. On the other hand, its desirable characteristics will also make it a novel platform for future biorefineries, which will make a significant contribution to green or sustainable chemistry (as shown in Figure 5).

Although extensive studies, such as general genetic tools, strategies of metabolic engineering, value-added bio-product production, genomic and transcriptomic, et cetera, have been developed in Z. mobilis since 1980s, non-commercialization of the Zymomonas process for ethanol production from sugar, starch-based or lignocellulosic biomass has developed successfully. Moreover, an increased range of higher-value product generation has also been restricted by its fundamental research. Especially, it is more difficult to engineer Z. mobilis than E. coli or yeast. Despite the extensive studies on general genetic tools and omics data available for Z. mobilis, it is necessary to further develop advanced technologies that can be used in metabolic engineering.

Therefore, to realize the industrial potential of Z. mobilis for future biorefineries, considerable efforts should be focused on the following points in the future: developing universal tools for deletion of several genes in one round, controlling metabolic flux and optimizing regulatory networks to improve the yield of desired products, and developing a highly express system, et cetera; these novel technologies are necessary for further strain improvement or redirection of the metabolic pathway for fuel and chemical production. Moreover, different systems of metabolic engineering approaches are becoming powerful tools in developing engineered E. coli or S. cerevisiae [3,5], which should also be highlighted in engineered Z. mobilis strains. In particular, other biotechnological approaches, such as genome sequencing, functional genomics, genome engineering and omics will also provide a basis for pathway or genome reconstruction to improve its fitness and robustness for environmental stress [160,161]. Representative biotechnologies, such as CRISPR/Cas systems [162], site-specific recombinases [163,164], genome shuffling [165], global transcription machinery engineering (gTME) [166], and Zinc-finger nucleases [167], which will also be used for enhancing cellular traits of Z. mobilis. Presumably, their potential will be further implemented with a promising future in developing or optimizing the metabolic pathway for the production of fuels as well as commodity and specialty chemicals.

Abbreviations
AcR: acetate-tolerant mutant; ALE: adaptive laboratory evolution; bp: base pairs; CBP: consolidated bioprocessing; CSTR: continuously stirred tank reactor; CTAB: cetyltrimethylammonium bromide; US DOE: US Department of Energy; ED pathway: Entner–Doudoroff pathway; GFOR: glucose-fructose oxidoreductase; GL: glucono-δ-lactone; gTME: global transcription machinery engineering; LBA: lactobionic acid; NGS: next-generation sequencing; NMR: nuclear magnetic resonance; subsp.: subspecies.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
This review was conceived, researched and written by M.X.H. BW and Z.YR participated in omic data collection and helped in manuscript editing. FRT summarized the section on strain improvement for Z. mobilis. JLW, ZXS, HQ, and QLZ summarized the section on succinic acid production by Z. mobilis and helped in management of references. LCD, XJT, and WGW participated in data collection and were involved in drafting the manuscript. KP and QCH participated in the discussion and helped in the draft manuscript editing. All authors read and approved the final manuscript.

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References
1. Lee JW, Na D, Park JM, Lee J, Choi S, Lee SY: Systems metabolic engineering of microorganisms for natural and non-natural chemicals. Nat Chem Biol 2012, 8:536–546.
2. Peralta-Yahya PP, Zhang F, Del Cardayre SB, Keasling JD: Microbial engineering for the production of advanced biofuels. Nature 2012, 488:320–328.
3. Chen XZ, Zhou L, Kangming T, Kumar A, Singh S, Pisor BA, Wang ZK: Metabolic engineering of Escherichia coli: a sustainable industrial platform for bio-based chemical production. Biotechnol Adv 2013, 31:1200–1223.
4. Nielsen J, Larsson C, van Maris A, Pronk J: Metabolic engineering of yeast for production of fuels and chemicals. Curr Opin Biotechnol 2013, 24:398–404.
5. Hong K-K, Nielsen J: Metabolic engineering of Saccharomyces cerevisiae: a key cell factory platform for future biorefineries. Cell Mol Life Sci 2012, 69:2671–2690.

6. Bentsen NS, Felby C: Biomass for energy in the European Union-a review of bioenergy resource assessments. Biotechnol Biofuels 2012, 5:1–10.

7. Zhou X, Wang F, Hu H, Yang L, Guo P, Xiao B: Assessment of sustainable biomass resource for energy use in China. Biomass Bioenergy 2011, 35:1–11.

8. Panesar PS, Marwaha SS, Kennedy JF: Zymomonas mobilis: an alternative ethanol producer. J Chem Technol Biotechnol 2006, 81:623–635.

9. Lee SY, Lee KJ, Kang HS, Oh J, Jin SJ, Um HW, Lee HJ, Oh SJ, Kim JR, Kang HL, Lee SY, Lee KJ, Kang HS: The genome sequence of the ethanologenic bacterium Zymomonas mobilis ZM4. Nat Biotechnol 2003, 2363–68.

10. Hong K-K, Nielsen J: Ethanol fermentation from biomass resources: current state and prospects. Appl Microbiol Biotechnol 2006, 69:627–642.

11. Rogers PL, Jeon YJ, Lee KJ, Lawford HG: Zymomonas mobilis strains revealing the existence of a novel subspecies Z. mobilis subsp. francensis nov., isolated from French cider. Int J Syst Evol Microbiol 2006, 56:121–125.

12. Coton M, Laplace JM, Coton E: Zymomonas mobilis subsp. identification by amplified ribosomal DNA restriction analysis. Lett Appl Microbiol 2005, 40:152–157.

13. Coton M, Laplace JM, Affray Y, Coton E: "Framebois" spoilage in French ciders: Zymomonas mobilis implications and characterization. J LWT-Food Sci Technol 2006, 39:972–979.

14. Jeon YJ, Park JM, Choi S, Choi YJ, Seung DY, Cho JH, Lee SY: Engineering of microorganisms for the production of biofuels and perspectives based on systems metabolic engineering approaches. Biotechnol Adv 2012, 30:899–1000.

15. Peng X, Park CJ, Oh HM, Lee JS, Jin SJ, Um HW, Lee HJ, Oh SJ, Kim JR, Kang HL, Lee SY, Lee KJ, Kang HS: Duplex PCR method for rapid identification of high acetate concentrations. J Biosci Bioeng 2013, 115:70–75.

16. Coton M, Laplace JM, Coton E: Zymomonas mobilis subsp. identification by amplified ribosomal DNA restriction analysis. Lett Appl Microbiol 2005, 40:152–157.

17. Coton M, Laplace JM, Coton E: Zymomonas mobilis subsp. identification by amplified ribosomal DNA restriction analysis. Lett Appl Microbiol 2005, 40:152–157.

18. Coton M, Laplace JM, Affray Y, Coton E: Duplex PCR method for rapid detection of Zymomonas mobilis in cider. J Inst Brew 2005, 111:299–303.

19. Swings J, Deleye J: Biology of Zymomonas. Bacteriol Rev 1977, 41:41–46.

20. Senthilkumar V, Rameshwar N, Busby S, Gunasekaran P: Disruption of the Zymomonas mobilis extracellular succrase gene (Sac) improves levan production. J Appl Microbiol 2004, 96:671–676.

21. Kerr AL, Jeon YJ, Svenson C, Rogers PL, Nellon BA: DNA restriction-modification systems in the ethanologens, Zymomonas mobilis ZM4. Appl Microbiol Biotechnol 2011, 89:761–769.

22. Wu B, He MX, Luo AJ, Zhang Y, Feng H, Hu QC, Zhang YZ: Construction and Characterization of restriction-modification deficient mutants in Zymomonas mobilis ZM4. Chin J Appl Environ Biol 2013, 19(2):189–197.

23. Jeon YJ, Park JM, Choi SY, Kim Jr, Kim JR: Method for mass production of primary metabolites, strain for mass production of primary metabolites, and method for preparation thereof. In: US Patent; 2007:20090162901 A1.

24. Viitanen PV, Tiao L, Knoke K, Zhang Y, Caiy G, Zhang MN, Mau Y-C, Franzen MA: Zymomonas with improved ethanol production in medium containing concentrated sugars and acetate. In: EP Patent; 2012:2208998 B1.

25. Viitanen PV, Tiao L, Knoke K, Zhang Y, Caiy G, Zhang MN, Mau Y-C, Franzen MA: Zymomonas with improved ethanol production in medium containing concentrated sugars and acetate. In: EP Patent; 2012:2208998 B1.

26. Viitanen PV, Tiao L, Knoke K, Zhang Y, Caiy G, Zhang MN, Mau Y-C, Franzen MA: Zymomonas with improved ethanol production in medium containing concentrated sugars and acetate. In: EP Patent; 2012:2208998 B1.

27. Viitanen PV, Tiao L, Knoke K, Zhang Y, Caiy G, Zhang MN, Mau Y-C, Franzen MA: Zymomonas with improved ethanol production in medium containing concentrated sugars and acetate. In: EP Patent; 2012:2208998 B1.

28. Viitanen PV, Tiao L, Knoke K, Zhang Y, Caiy G, Zhang MN, Mau Y-C, Franzen MA: Zymomonas with improved ethanol production in medium containing concentrated sugars and acetate. In: EP Patent; 2012:2208998 B1.

29. Viitanen PV, Tiao L, Knoke K, Zhang Y, Caiy G, Zhang MN, Mau Y-C, Franzen MA: Zymomonas with improved ethanol production in medium containing concentrated sugars and acetate. In: EP Patent; 2012:2208998 B1.

30. Viitanen PV, Tiao L, Knoke K, Zhang Y, Caiy G, Zhang MN, Mau Y-C, Franzen MA: Zymomonas with improved ethanol production in medium containing concentrated sugars and acetate. In: EP Patent; 2012:2208998 B1.

31. Viitanen PV, Tiao L, Knoke K, Zhang Y, Caiy G, Zhang MN, Mau Y-C, Franzen MA: Zymomonas with improved ethanol production in medium containing concentrated sugars and acetate. In: EP Patent; 2012:2208998 B1.

32. Viitanen PV, Tiao L, Knoke K, Zhang Y, Caiy G, Zhang MN, Mau Y-C, Franzen MA: Zymomonas with improved ethanol production in medium containing concentrated sugars and acetate. In: EP Patent; 2012:2208998 B1.

33. Viitanen PV, Tiao L, Knoke K, Zhang Y, Caiy G, Zhang MN, Mau Y-C, Franzen MA: Zymomonas with improved ethanol production in medium containing concentrated sugars and acetate. In: EP Patent; 2012:2208998 B1.

34. Viitanen PV, Tiao L, Knoke K, Zhang Y, Caiy G, Zhang MN, Mau Y-C, Franzen MA: Zymomonas with improved ethanol production in medium containing concentrated sugars and acetate. In: EP Patent; 2012:2208998 B1.
In Biotechnology for Fuels and Chemicals, edited by Finkelman M, McClellan J, Davison B, Springer, Appl Biochem Biotechnol 2002:98–100, 899–907.

50. Skotnicki ML, Lee K, Tribe D, Rogers P: Genetic alteration of Zymomonas mobilis for ethanol production. In Genetic Engineering of Microorganisms for Chemicals, edited by Hollaender A, DeMoss RD, Kaplan S, Konisky J, Savage D, Wolfe RS, New York: Plenum Press, 1982:221–290.

51. Carey V, Walla S, Ingraham L: Expression of a lactose transposon (Tn51) in Zymomonas mobilis. Appl Environ Microbiol 1983, 46:1163–1168.

52. Carey V, Walla S, Ingraham L: Expression of a lactose transposon (Tn51) in Zymomonas mobilis. Appl Environ Microbiol 1983, 46:1163–1168.

53. Buchholz SE, Eaveleigh DE: Genetic modification of Zymomonas mobilis. Biotechnol Adv 1990, 8:547–581.

54. Pappas KM, Galeros M, Pappas K-M, Beletsiotis E, Typas MA: ISZm1068: an IS5-like insertion element from Zymomonas mobilis. Arch Microbiol 2001, 175:323–333.

55. Chatterjee R, Yuan L: Adaptation yields a highly efficient Escherichia coli strain for xylose and arabinose fermentation. Biotechnol Adv 2013, 31:1294.

56. Dragosits M, Mattanovich D: Adaptive laboratory evolution—principles and applications for biotechnology. Microbiol Cell Fact 2013, 12:58.

57. Hua Q, Joyce AR, Palsson BO: Adaptive evolution of an industrial strain of Zymomonas mobilis. Biotechnol Lett 2010, 32:459–594.

58. Lee D-H, Palsson BO: Adaptive evolution of Escherichia coli K-12 MG1655 and temperature. Saccharomyces cerevisiae J Appl Microbiol 2010, 12:331–336.

59. Demeke MM, Dietz H, Li Y, Foulquié-Moreno MR, Mutturi S, Deprez S, Den Abt T, He M-X, Feng H, Li Y, Bai F, Liu X, Zhang Y-Z, Kim IS, Barrow KD, Rogers PL: Nuclear magnetic resonance studies of acetic acid inhibition of rec Zymomonas mobilis ZM4 (pZB5). Appl Microbiol Biotechnol 2002, 98:100885–898.

60. Ruanglek V, Maneewatthana D, Tripetchkul S: Isolation and preliminary characterization of a Zymomonas mobilis mutant with an altered preference for xylose and glucose utilization. Biotechnol Lett 2000, 22:157–164.

61. Mohagheghi A, Linger J, Smith H, Yang S, Downe N, Penkosk P: Improving xylose utilization by recombinant Zymomonas mobilis strain 8b through adaptation using 2-deoxyglucose. Biotechnol Biofuels 2014, 7:191.

62. Agrawal M, Chen RR: Discovery and characterization of a xylose reductase from Zymomonas mobilis. J Appl Microbiol 2010, 108:777–85.

63. Agrawal M, Mao Z, Chen RR: Adaptation yields a highly efficient xylose-fermenting and inhibitor tolerant industrial Saccharomyces cerevisiae strain with high performance in lignocellulosic hydrolysates using metabolic and evolutionary engineering. Biotechnol Biofuels 2013, 6:69.

64. Dhar R, Sägesser R, Wei kert C, Yuan J, Wagner A: Adaptation of Saccharomyces cerevisiae on stress conditions through evolutionary laboratory. J Evol Biol 2011, 24:1135–1153.

65. Wang Y: Development of acetic-acid tolerant Zymomonas mobilis strains through adaptation. In Master Thesis. Georgia Institute of Technology, Chemical Engineering, 2008. URL: http://hdl.handle.net/1853/29747.

66. Agrawal M, Mao Z, Chen RR: Adaptation yields a highly efficient xylose-fermenting Zymomonas mobilis strain. Biotechnol Bioeng 2011, 108:777–785.

67. He M-X, Feng H, Li Y, Bai F, Liu X, Zhang Y-Z: Direct production of ethanol from raw sweet potato starch using genetically engineered Zymomonas mobilis. Afr J Microbiol Res 2009, 3:271–276.

68. He M-X, Li Y, Liu X, Bai F, Feng H, Zhang Y-Z: Ethanol production by mixed-cultures of Paenibacillus sp and Zymomonas mobilis using the raw stalk material from sweet potato. Ann Microbiol 2009, 59:749–754.

69. Schmier KP, MR, Mitchell RB, Perret NW: Net energy of cellulolic ethanol from switchgrass. Proc Natl Acad Sci US A 2008, 105:464–469.

70. Pimentel D, Patzek T: Ethanol production using corn, switchgrass, and wood; biodiesel production using soybean and sunflower. Nat Resour Res 2005, 14:65–76.

71. Balat M, Balat H: Recent trends in global production and utilization of bio-ethanol fuel. Appl Energy 2009, 86:2273–2282.

72. Zhang M, Eddy C, Deanda K, Finkelman M, Picataqio S: Metabolic engineering of a pentose metabolism pathway in ethanologenic Zymomonas mobilis. Science 1995, 267:240–243.

73. Deanda K, Zhang M, Eddy C, Picataqio S: Development of an arabino-fermenting Zymomonas mobilis strain by metabolic pathway engineering. Appl Environ Microbiol 1996, 62:4465–4470.

74. Mohagheghi A, Evans K, Finkelman M, Zhang M: Cofermentation of glucose, xylose, and arabinose by mixed cultures of two genetically engineered Zymomonas mobilis strains. Appl Biochem Biotechnol 1998, 70–72:285–299.

75. Zhang M, Zhou Y-C, Picataqio S, Finkelman M: Single Zymomonas mobilis strain for xylose and arabinose fermentation. U.S. Patent; 1998:5845760.

76. Mohagheghi A, Evans K, Zhou Y-C, Zhang M: Cofermentation of glucose, xylose, and arabinose by genomic DNA-integrated xylose/arabinose fermenting strain of Zymomonas mobilis AX101. Appl Biochem Biotechnol 2002, 98–100:885–898.

77. Lawford HG, Rousseau JD: Improving fermentation performance of recombinant Zymomonas mobilis in acetic acid-containing media. Appl Biochem Biotechnol 1998, 70–72:161–172.

78. Jeon YJ, Svenson CJ, Rogers PL: Over-expression of xyloolkinase in a xylose-metabolising recombinant strain of Zymomonas mobilis. FEMS Microbiol Lett 2005, 244:1–52.

79. A. Cakar ZP, Turanli AS, Bocholtz E, Dunn NW, Rogers PL: Isolation and preliminary characterization of a Zymomonas mobilis mutant with an altered preference for xylose and glucose utilization. Biotechnol Lett 2000, 22:157–164.

80. Mohagheghi A, Linger J, Smith H, Yang S, Downe N, Penkosk P: Improving xylose utilization by recombinant Zymomonas mobilis strain 8b through adaptation using 2-deoxyglucose. Biotechnol Biofuels 2014, 7:191.

81. Lawford HG, Rogers PL: Genetic alteration of Zymomonas mobilis ZM4 for ethanol production from sugar cane bagasse by Zymomonas mobilis using simultaneous saccharification and fermentation (SSF) process. Appl Biochem Biotechnol 2010, 161:93–105.

82. Su R, Ma Y, Q, W, Zhang M, Wang F, Du R, Yang J, Zhang M, He Z: Ethanol production from high-solids SSCF of alkaline-pretreated corn cob using recombinant Zymomonas mobilis ZP, Bioenergy Res 2010, 3:299.

83. Linger JG, Adney WS, Darzins A: Heterologous expression and extracellular secretion of cellulolytic enzymes by Zymomonas mobilis. J Appl Microbiol 2010, 117:211–217.

84. Lynd LR, Zyl WH, McBride JE, Laser M: Consolidated Bioprocessing. J Appl Microbiol 2000, 89:577–583.

85. Lawford HG, Rousseau JD: Comparative ethanol productivities of different Zymomonas recombinants fermenting oak hull hydrolysate. Appl Biochem Biotechnol 2001, 91:133–146.

86. Su R, Ma Y, Q, W, Zhang M, Wang F, Du R, Yang J, Zhang M, He Z: Ethanol production from bamboo residues with lignocellulose fractionation using simultaneous saccharification and fermentation (SSF) process. Appl Biochem Biotechnol 2010, 161:93–105.

87. Linger JG, Darzins A: Heterologous expression and extracellular secretion of cellulolytic enzymes by Zymomonas mobilis. J Appl Microbiol 2010, 117:211–217.

88. Lynd LR, Zyl WH, McBride JE, Laser M: Consolidated Bioprocessing of lignocellulosic biomass: an update. J Appl Microbiol 2005, 105:577–583.

89. Linger JG, Adney WS, Darzins A: Heterologous expression and extracellular secretion of cellulolytic enzymes by Zymomonas mobilis. J Appl Microbiol 2010, 117:211–217.
The simultaneous production of sorbitol from fructose and gluconic acid from glucose using an oxidoreductase of Zymomonas mobilis, isolated from permeabilized cells of Zymomonas mobilis by untreated cells of Zymomonas mobilis. J Biol Chem 1999, 75:99–103.

Cazetta ML, Celligoi MAPC, Buzatto JB, Scarrino IS, da Silva RSF: Optimization study for sorbitol production by Zymomonas mobilis in sugar cane molasses. Process Biochem 2005, 40:747–751.

Liu C, Dong HW, Zhong J, Ryu DD, Bao J: A proposed pathway for sorbitol production by Zymomonas mobilis in batch and continuous fermentation systems. Carbohydr Polym 2014, 99:544–546.

Beauprez JJ, De Mey M, Soetaert WK: Microbial succinic acid production: natural versus metabolic engineered producers. Process Biochem 2010, 45:1103–1114.

Lee S, Lee D-Y, Kim TY, Kim BH, Lee J, Lee SY: Metabolic engineering of Escherichia coli for enhanced production of succinic acid, based on genome comparison and in silico gene knockout simulation. Appl Microbiol Biotechnol 2003, 71:788–7887.

Jiang M, Liu SW, Ma J, Chen K, Yu L, Yue F, Xu B, Wei P: Effect of growth phase feeding strategies on succinate production by metabolically engineered Escherichia coli. Appl Environ Microbiol 2010, 76:1298–1300.

Agrin R, Otero JM, Nielsen J: Genome-scale modeling enables metabolic engineering of Saccharomyces cerevisiae for succinic acid production. J Ind Microbiol Biotechnol 2013, 40:735–747.

Sobczak AM, Gebhardt H, Bultoza N, Weuster-Botz D, Lang C: Metabolic engineering of Saccharomyces cerevisiae for the biotechnological production of succinic acid. Metab Eng 2010, 12:518–525.

Lee K, Park J, Kim T, Yun H, Lee S: Genome-scale metabolic network analysis of Zymomonas mobilis ZM4 explains physiological features and suggests ethanol and succinic acid production strategies. Microcell Fact 2010, 9:94.

Widiatni H, Kim JY, Seharasoe, Karim IA, Kim H, Seo JS, Lee DY: Genome-scale modeling and in silico analysis of ethanologenic bacteria Zymomonas mobilis. Biotechnol Bioeng 2011, 108:655–665.

Pentjusa A, Ogtina I, Kostormins A, Fell D, Stalidzans E, Kalnenieks U: Biotechnological potential of respiring Zymomonas mobilis: a stoichiometric analysis of its central metabolism. J Biotechnol 2013, 165:1–10.

Atsumi S, Hanai T, Liao JC: Non-fermentative pathways for synthesis of branched-chain higher alcohols as biofuels. Nature 2008, 45386–89.

Atsumi S, Wu TY, Eckl EM, Hawkins SD, Buelter T, Liao JC: Engineering the isobutanol biosynthetic pathway in Escherichia coli by comparison of three aldehyde reductase/alkalcohol dehydrogenase genes. Appl Environ Microbiol 2010, 85:651–657.

Atsumi S, Liao JC: Metabolic engineering for advanced biofuels production from Escherichia coli. Curr Opin Biotechnol 2008, 19:414–419.

Chen X, Nielsen KF, Borodina I, Kielland-Brandt MC, Kathuma K: Increased isobutanol production in Saccharomyces cerevisiae overexpression of genes in valine metabolism. Biotechnol Biofuels 2011, 4:2099–2900.

Brat D, Boles E: Isobutanol production from D-ylose by recombinant Saccharomyces cerevisiae. FEBS Yeast Res 2013, 3:241–244.

Brundard P, Lorgo V, Berterame NM, Rosi G, Porro D: A novel pathway to produce butanol and isobutanol in Saccharomyces cerevisiae. Biofuels Biotechnol 2013, 6:1–12.

Smith KM, Cho K-M, Liao JC: Engineering Corynebacterium glutamicum for isobutanol production. Appl Microbiol Biotechnol 2010, 87:1045–1055.

Blomback B, Riester T, Wieschalla S, Zient C, Youn J-W, Wendisch VF, Ekmanns Bj: Corynebacterium glutamicum tailored for efficient isobutanol production. Appl Environ Microbiol 2011, 77:3300–3310.
147. Yamamoto S, Suda M, Niami S, Inui M, Yukawa H: Strain optimization for efficient isobutanol production using Corynebacterium glutamicum under oxygen deprivation. Biotechnol Bioeng. 2013, 110(2):2938–2948.

148. Li SS, Wen JP, Jia XQ: Engineering Bacillus subtilis for isobutanol production by heterologous Ehrlich pathway construction and the biosynthetic 2-ketoisovalerate precursor pathway overexpression. Appl Microbiol Biotechnol. 2011, 91:577–589.

149. Minty JJ, Singer ME, Scholz SA, Bae C-H, Ahn J-H, Foster CE, Liao JC, Lin XN: Design and characterization of synthetic fungal-bacterial consortia for direct production of isobutanol from cellullosic biomass. Proc Natl Acad Sci U S A. 2013, 110:14592–14597.

150. Uhlenbusch I, Sahm H, Sprenger GA: Expression of an L-alanine dehydrogenase gene in Zymomonas mobilis and excretion of L-alanine. Appl Environ Microbiol. 1991, 57:1360–1366.

151. Keasling JD: Manufacturing molecules through metabolic engineering. Science 2010, 330:1355–1358.

152. Maury J, Asadollahi M, Møller K, Clark A, Nielsen J: Manufacturing molecules through metabolic engineering. In: Biotechnology for the Future, Volume 100. Edited by Nielsen J. Berlin Heidelberg: Springer, 2005:19–51. Advances in Biochemical Engineering/Biotechnology.

153. Hermans MA, Neuss B, Sahm H: Content and composition of hopanoids in Zymomonas mobilis under various growth conditions. J Bacteriol. 1991, 173:599–5995.

154. Schmidt A, Bringer-Meyer S, Poralla K, Sahm H: Effect of alcohols and temperature on the hopanoid content of Zymomonas mobilis. Appl Microbiol Biotechnol. 1986, 25:32–36.

155. Charon L, Pale-Grosdemange C, Rohmer M: On the reduction steps in the mevalonate independent 2-C-methyl-d-erythritol 4-phosphate (MEP) pathway for isoprenoid biosynthesis in the bacterium Zymomonas mobilis. Tetrahedron Lett. 1999, 40:7231–7234.

156. Perzl M, Reipen IJ, Schmitz S, Poralla K, Sahm H, Sprenger GA, Elmar L: Cloning of conserved genes from Zymomonas mobilis and Bradyrhizobium japonicum that function in the biosynthesis of hopanoid lipids. Biochim Biophys Acta (BBA) - Lipids Lipid Metabol. 1998, 1393:108–118.

157. Grolle S, Bringer-Meyer S, Sahm H: Isolation of the dxr gene of Zymomonas mobilis and characterization of the 1-deoxy-D-xylulose 5-phosphate reductoisomerase. FEMS Microbiol Lett. 2000, 191:131–137.

158. Vincent SP, Sinay P, Rohmer M: Composite hopanoid biosynthesis in Zymomonas mobilis: N-acetyl-D-glucosamine as precursor for the cyclopentane ring linked to bacteriohopanetetrol. Chem Commun. 2003, 6:782–783.

159. Misawa N, Yamano S, Ikenaga H: Production of beta-carotene in Zymomonas mobilis and Agrobacterium tumefaciens by introduction of the biosynthesis genes from Envinia uredovora. Appl Environ Microbiol. 1991, 57:1847–1849.

160. Wang HH, Isaacs FJ, Carr PA, Sun ZZ, Xu G, Forest CR, Church GM: Programming cells by multiplex genome engineering and accelerated evolution. Nature 2009, 460:894–906.

161. Carr PA, Church GM: Genome engineering. Nat Biotechnol 2009, 27:1151–1162.

162. Cong L, Ran FA, Cox D, Lin S, Barretto R, Habib N, Hsu PD, Wu X, Jiang W, Marraffini LA: Multiplex genome engineering using CRISPR/Cas systems. Science 2013, 339:819–823.

163. Kolb AF: Genome engineering using site-specific recombinases. Cloning Stem Cells. 2002, 4:65–80.

164. Wirther D, Gama-Norton L, Riemer P, Sandhu UJ, Schucht R, Hauser H: Road to precision: recombinase-based targeting technologies for genome engineering. Curr Opin Biotechnol. 2007, 18:411–419.

165. Zhang YX, Perry K, Vinci VA, Powell K, Stemmer WP, del Cardayre SB: Genome shuffling leads to rapid phenotypic improvement in bacteria. Nature 2002, 415:646–646.

166. Alper H, Stephanopoulos G: Global transcription machinery engineering: a new approach for improving cellular phenotype. Metab Eng. 2007, 9:258–267.

167. Carroll D: Genome engineering with zinc-finger nucleases. Genetics 2011, 187:773–782.