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Acetate as a potential feedstock for the production of value-added chemicals: Metabolism and applications

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[1] Abbreviations

Abstract:

Acetate is regarded as a promising carbon feedstock in biological production owing to its possible derivation from C_1 gases such as CO, CO_2 and methane. To best use of acetate, comprehensive understanding of acetate metabolisms from genes and enzymes to pathways and regulations is needed. This review aims to provide an overview on the potential of acetate as carbon feedstock for industrial biotechnology. Biochemical, microbial and biotechnological aspects of acetate metabolism are described. Especially, the current state-of-the art in the production of value-added chemicals from acetate is summarized. Challenges and future perspectives are also provided.

Keywords:

Acetate; Value-added chemicals; Metabolism; Regulations; Tolerance

[1] AAB; acetic acid bacteria; ACS, acetyl-CoA synthetase; ALE, adaptive laboratory evolution; AT, active transport; CAD, cis-aconitate decarboxylase; CFA, cyclopropane fatty acid; CRP, cyclic AMP receptor protein; CS, citrate synthase; DOE, Department of Energy; ETC, electron transport chain; FA, fatty acids; FNR, fumarate and nitrate reductase; GABA, gamma-aminobutyric acid; IA, itaconic acid; ICL, isocitrate lyase; IPP, isopentyl pyrophosphate; MA, mevalonic acid; MCR, malonyl-CoA reductase; MS, malate synthase; PFL, pyruvate-formate lyase; PEP, phosphoenolpyruvate; PMCT, proton-linked monocarboxylate transporter; SATP, succinate-acetate transporter protein; SCS, succinyl-CoA synthetase; SSS, solute:sodium symporter; TCA, tricarboxylic acid; TRY, titer, rate, and yield; VFA, volatile fatty acids

1. INTRODUCTION

The non-renewable nature of fossil resources and the environmental concerns about global warming have encouraged the use of eco-friendly resources for biofuel production. The success of this sustainable production technology largely depends on the efficiency of microbial cell factories (Chung et al., 2015; Seo et al., 2013) and the cost of carbon feedstock (Lim et al., 2018; Novak and Pflugl, 2018). The first generation of biofuel technologies, which are currently under operation, was developed on edible feedstocks such as glucose, sucrose, starch, vegetable oil, etc., and their competition with the human food chain has been criticized. Consequently, the replacement of current feedstocks with less expensive and non-edible ones is demanded (Kumar et al., 2013; Kumar and Park, 2018; Lim et al., 2018).

Acetic acid, a two-carbon monocarboxylic acid (CH_3COOH) with a molecular mass of 60 g/mol, is emerging as an alternative feedstock for biofuel production. Its pK_a value is 4.75; thus, acetic acid is predominantly dissociated as acetate (CH_3COO^-) at physiological pH. The global production of acetate is approximately 12.9 million metric tons each year, priced at $350–450/ton, which is slightly cheaper than conventional sugars such as glucose, which is priced at $500/ton (Li et al., 2016; Lim et al., 2018). Acetate can be produced using both chemical and biological methods (**Fig. 1**). Chemically, acetate can be synthesized via methanol carbonylation, ethylene oxidation, or alkane oxidation, accounting for about 75% of the total acetate production. Biologically, acetate can be formed from sugars, glycerol, lignocellulosic biomass, or even waste materials, mostly by *Acetobacter* species. Acetate can also be produced from C_1 gases such as CO and CO_2 by acetogenic bacteria via the autotrophic Wood-Jungdahl pathway (Hu et al., 2016; Schiel-Bengelsdorf and Durre, 2012; Schuchmann and Muller, 2014). Bacterial CO/CO_2 assimilation is considerably more efficient than photosynthetic assimilation, and this technology, once developed, is expected to be an effective solution to the global warming caused by C_1 gases. As the capture or immobilization of C_1 gases and their conversion to acetate needs to be conducted on a massive scale, acetate has the potential to become the most abundant carbon feedstock in the future. Furthermore, acetate can empower the development of low-cost, sustainable bioprocesses, without any conflicts with arable land and the food chain (Lim et al., 2018; Novak and Pflugl, 2018) (**Fig. 1**).

The current review focuses on the potential of acetate as a feedstock for industrial biotechnology. First, the biochemical aspects of acetate production and utilization, such as the enzymes, pathways, transport, and regulation of the genes and enzymes involved, are described. Second, the microbial aspects of acetate utilization, including growth, adaptation, and tolerance, are described. Third, the biotechnological potential of acetate as a carbon feedstock is summarized for various products such as succinic acid, itaconic acid, 3-hydroxypropionic acid, fatty acids, microbial lipids, isobutyl acetate, mevalonate, β-caryophyllene, polyhydroxyalkanoate, etc. Finally, the challenges and perspectives of acetate utilization as a carbon source are described. This review provides an overview of the current status of acetic acid utilization for biotechnological purposes.

2. METABOLISM OF ACETATE IN MICROORGANISMS

The optimal use of acetate as a carbon feedstock requires an understanding of its metabolism, including the enzymes involved and the regulation of these enzymes and gene expression. Generally, acetate is directed to major metabolic pathways through acetyl coenzyme A (acetyl-CoA), an activated form of acetate. Subsequently, the two-carbon acetate is converted to higher carbon compounds such as C_5 ribose and C_6 glucose through the glyoxylate pathway and gluconeogenesis. Several outstanding review papers have been published on acetate metabolism, including one by Bernal et al. (2016), which mainly deals with acetate production during heterotrophic growth. In this section, acetate assimilation, the glyoxylate shunt, acetate transporters, and their regulation are described from the aspect of acetate utilization (**Fig. 2**). Acetate formation from acetyl-CoA and pyruvate is also briefly described owing to its close association with the acetate assimilation pathways and the regulation of expression of genes regarding acetate metabolism.

2.1 Acetate assimilation and conversion to acetyl-CoA

The conversion of acetate to acetyl-CoA is the first step of acetate assimilation and can be performed via the acetate kinase-phosphotransacetylase (ACKA-PTA) and/or acetyl-CoA synthetase (ACS) pathways. The ACKA-PTA pathway is reversible, enabling bidirectional conversion between acetate and acetyl-CoA, whereas the ACS pathway is irreversible, involving enzyme-bound acetyl-AMP as an intermediate (Lin et al., 2006) (**Fig. 2**). Although both pathways consume one adenosine triphosphate (ATP) molecule to activate acetate to acetyl-CoA, the ACS pathway is energetically more expensive since the pyrophosphate (PP_i) formed along with acetyl-AMP is further split into two P_i.

In the ACKA-PTA pathway, ACKA (acetate kinase; EC 2.7.2.1) reversibly transfers the phosphate group from ATP to acetate to form acetyl phosphate (acetyl-P_i) and ADP (adenosine diphosphate). Subsequently, PTA (acetyl-CoA:P_i acetyltransferease; EC 2.3.1.8) condenses acetyl-P_i and CoA-SH into acetyl-CoA with the release of an inorganic phosphate (Dittrich et al., 2005a; Shi et al., 2005). The reverse reaction of acetate formation, i.e., the production of acetate from acetyl-CoA, which occurs when heterotrophic bacteria grow on organic carbon such as glucose or when acetogenic bacteria grow on C_1 compounds and produce acetate from via Wood-Ljundahl pathway, has been extensively studied. Especially during anaerobic microbial growth, ATP generation by this reverse reaction (substrate-level phosphorylation) is critical for cell growth (Dittrich et al., 2005a; Dittrich et al., 2005b; Hasona et al., 2004). Similarly, the acetate assimilation to acetyl-CoA by acetyl-CoA synthetase (acetate:CoA ligase [AMP forming]; EC 6.2.1.1) also proceeds in two steps, but without release of acetyl-AMP as an intermediate. In the first step, the enzyme produces the acetyl-AMP intermediate (enzyme-bound) from acetate and ATP, with the release of a PP_i molecule. In the second step, the acetyl-AMP-enzyme complex reacts with CoA to produce acetyl-CoA and AMP. ACS belongs to the AMP-forming enzyme family, which also includes the firefly luciferase and non-ribosomal peptide synthetases (Jogl and Tong, 2004). ACS functions anabolically and scavenges environmental acetate at low concentrations in heterotrophic bacteria including *E. coli* and *Samonella typhimurim* (Brown et al., 1977; Kumari et al., 1995). It also plays an important role in acetoclastic methanogens which mostly belong to thermophilic archaea (Ingram-Smith et al., 2005; Ingram-Smith et al., 2007).

In *E. coli*, the PTA-ACKA pathway operates during either anaerobic or aerobic growth on glucose (acetate overflow). The two genes, *pta* and *ackA*, are

constitutively expressed from the same operon, although *ackA* is known to be positively regulated by FNR (fumarate nitrate reductase regulator) (Wanner et al., 1992; Shalel-Levanon et al., 2005). The intermediate, acetyl-P_i, is energetically unstable and can phosphorylate regulatory proteins involved in two-component signal transduction. It controls flagella synthesis (CheY-CheA) and cell division, and is involved in regulation of phosphate concentration (PhoB-PhoR) (Kumari et al., 2000). Also, acetyl-P_i can acetylate lysine residue of proteins as post-translational modification, which affect function and activity of the acetylated proteins (Weinert et al., 2013). The *acs* gene is transcribed from a polycistronic operon together with two other genes, *yjcH*, an inner membrane conserved protein, and *actP*, which encodes an acetate permease. The *acs* expression is regulated by global and local regulatory proteins (CRP, FNR and IclR), nucleoid-associated proteins (FIS and IHF), two sigma factors (σ^{70} and σ^{S}), and two promoters (P2 as a primary promoter; P1 as a weak, secondary promoter located about 200 bps upstream of P2) (Wolfe, 2005). Furthermore, at protein level, enzymatic activity of ACS is highly regulated by acetylation (Starai et al., 2002; Starai et al., 2004; Starai et al., 2005; Castaño-Cerezo et al., 2011). Kumari et al. (1995) showed cell growth profiles of *E. coli* mutants on acetate: although wild-type *E. coli* grew well at the wide range of concentrations (2.5 to 50 mM), the *acs*-deficient mutant grew poorly at low concentrations (≤ 10 mM), the *pta-ackA* deficient mutant grew poorly at high concentrations (≥ 25 mM), and the mutant deleted for both *acs* and *pta-ackA* did not grow at all on acetate. The different growth at varying acetate concentrations suggests that having both PTA-ACKA and ACS are beneficial for growth on acetate.

Kinetic properties of the ACKA and ACS enzymes to acetate have been reported in many microorganisms which include heterotrophic bacteria (Gram-positive and Gram-negative bacteria including *E. coli*, *S. typhimurim*, *Corynebacterium glutamicum*, *Lactobacillus*, etc.), acetogens (anaerobic; *Clostridia sp.*), and acetoclastic/acetotrophic methanogens (mostly thermophilic archaea; *Methanosarcina*, *Methanothermobacter,* etc.) (**Table 1**). One yeast, *Saccharomyces cerevisiae*, was also studied for ACS. We notice that, in many heterotrophic bacteria, ACKA showed higher affinity to acetyl-P_i than acetate, which suggests that, physiologically, this enzyme is more important for acetate production rather than its consumption. In addition, ACS showed higher affinity to acetate than ACKA; the K_M values of ACKA were 7 ~ 300 mM, while those of ACS were 0.2 ~ 8.8 mM. One exception is found in *S. typhimurium*; K_M's for both enzymes were comparable as K_M (ACKA) = 1.2 or 7 mM and K_M (ACS) = 6.05 mM, respectively. However, to fully discuss the importance of different K_M values in different microorganisms, the reliability of the reported values should be carefully examined. In fact, the reported K_M values of both ACKA and ACS vary in a wide range, by ~400-fold difference. The wide range can be caused by difference in enzymes (i.e., amino acid sequences and/or 3D structures) present in diverse microbial strains, but the discrepancy of assay methods/conditions and/or errors in determination cannot be fully ignored. Experimental error can be reduced if measurement is repeated multiplication. However, the cases where K_M was determined more than once are very scarce and, furthermore even in the cases where determination was made more than twice (but by different groups), the values are not similar. For example, in *E. coli*, two reported K_M values of ACKA were ~8.4-fold different as: 7 mM (Fox and Roseman, 1986) and 59 mM (Nakajima et al., 1978). Similarly, in *S. typhimurium*, two reported K_M values of ACKA were ~5.8-fold different as: 1.2 mM (Chittori et al., 2012) and 7 mM (Fox

and Roseman, 1986). As another case, for thermophilic archaeon *Methanosarcina thermophile,* two reported K_M values of ACKA were 14.7-fold different as: 1.5 mM (Ingram-Smith et al., 2005) and 22 mM (Aceti et al., 1988). In acetogenic *Clostridium acetobutylicum*, two K_M values of ACKA were 2-fold different as: 73 mM (Diez-Gonzalez et al., 1996) and 160 mM (Winzer et al., 1997). For ACS, no multiple measurements on K_M values have been reported. As an exception, for ACS of *S. typhimurium*, another measurement (K_M = 40 mM) has been reported but the measurement has been conducted with the ACS purified from a mutant *S. typhimurium* which is *cobB*$^-$*patZ*$^-$ (Chan et al., 2011). The mutation in *cobB* and/or *patZ* has been suggested to give different post-translational acetylation to the ACS protein (see Section 2.4 **Regulation of acetate metabolism),** thus alter kinetic property of the enzyme.

Comparison between *E. coli* and *S. typhimurium* needs some attention. Their ACKA and ACS have been studied more extensively than in other microorganisms. For ACKA, if we ignore the high K_M of *E. coli* (59 mM), the rest three K_M values for *E. coli* and *S. typhimurium* are in the same order of magnitude, 1.2 ~ 7 mM. In fact, one group (Fox and Roseman, 1986) determined K_M values of ACKA in the two microorganisms in one study and reported that they are the same as 7 mM. We found that the amino acid sequences of ACKA of *E. coli* and *S. typhimurium* are 98% similar. Therefore, kinetic properties of ACK of both the strains are expected to be quite similar. This suggests that the high K_M value of ACKA (59 mM) of *E. coli* reported by Nakajima et al. (1978) might be less reliable. If the high K_M of *E. coli* (59 mM) is ignored, it is suggested that ACKA of *E. coli* and *S. typhimurium* have generally higher affinity than most other ACKA, except that of *Corynebacterium glutamicum* and the acetoclastic/acetotrophic methanogen, *Methanosarcina thermophile* (Ingram-Smith et al., 2005; Ferry et al., 1997). This indicates that, in these microorganisms, ACKA has been evolved to catalyze acetate-consuming reaction more efficiently than in other microorganisms, especially in comparison to *Moorella thermoacetica, Clostridium thermocellum*, and *Clostridium acetobutylicum*. The latter three microorganisms are acetogenic and high affinity of their ACKA to acetate should not be necessary or even disadvantageous for their growth. For ACS, we find that *E. coli* (0.2 mM) has lower K_M than *S. typhimurium* (6.05 mM). It should be more appropriate to say that *E. coli*'s ACS has an exceptionally lower K_M in comparison to ACS of many other bacteria and archaea (see **Table 1**). However, similarity of amino acid sequences of ACS between *E. coli* and *S. typhimurium* is >90%. It is widely recognized that *S. typhimurium* is very close to *E. coli* K12, sharing about 85% of genome of the latter (Sargo et al., 2015; AbuOun et al., 2009; McClelland et al., 2001). Interestingly, comparative study between *S. typhimurium* and *E. coli* has shown that acetate uptake rate as well as the maximum growth rate were significantly lower in *S. typhimurium* (3-fold and 2.1-fold, respectively). The lower acetate uptake in *S. typhimurium* can be partly caused by the lower affinity of its ACS than that of *E. coli*.

In *Saccharomyces cerevisiae*, two ACSs, ACS1 and ACS2, showing different affinities toward acetate as 0.32 mM (ACS1) and 8.8 mM (ACS2), respectively, have been reported. (van den Berg et al., 1996; Takahashi et al., 2006). Knockout mutants where either ACS1 or ACS2 is disrupted have been constructed to study their physiological roles (van den Berg et al., 1996). ACS1 played a key role in assimilating of acetate or ethanol and was repressed by glucose. The enzyme was located in peroxisome along with the glyoxylate shunt enzymes (citrate synthase,

isocitrate lyase, malate synthase and malate dehydrogenase) (van den Berg et al., 1995), indicating that ACS1 is primarily responsible for acetate activation as ACSs in many bacteria. ACS1 had a high similarity in gene sequence with ACSs of several microorganisms, including *Aspergillus nidulans* (62.4%; Connerton et al., 1990), *Penicillium chrysogenum* (61.2%; Martinez-Blanco et al., 1993), *E. coli* (48.7%; Blattner et al., 1993), and *Methanothrix soehngenii* (44.6%; Eggen et al., 1991) (van den Berg et al., 1995). In contrast, ACS2 was important for growth on glucose and regulated global gene transcription via histone acetylation (van den Berg et al., 1996; van den Berg et al., 1995; Takahashi et al., 2006). The amino acid sequences of ACS1 and ACS2 have 76.3% similarity and 57% identity (van den Berg et al., 1995).

The microbial production of acetate from pyruvate occurs mostly via acetyl-CoA. Pyruvate formate lyase (PFL; encoded by *pflB*) and the pyruvate dehydrogenase complex (PDHc; encoded by *aceEF*) play major roles in the conversion of pyruvate to acetyl-CoA under anaerobic and aerobic conditions, respectively. The formation of acetate under aerobic conditions is the result of “overflow metabolism” meaning that pyruvate (or acetyl-CoA) production occurs faster than its consumption by the tricarboxylic acid (TCA) cycle. However, once glucose is completely depleted, the acetate generated by overflow metabolism is re-assimilated as a carbon and energy source. This indicates that the production and re-assimilation of acetate under aerobic conditions are closely linked to each other and stringently controlled to meet the cellular demand. Under aerobic conditions, acetate is also formed directly from pyruvate via oxidative decarboxylation catalyzed by pyruvate oxidase (POXB), which is activated in the early stationary phase of cell growth (De Mey et al., 2007). In general, PTA-ACKA is the major pathway for acetate formation (Enjalbert et al., 2017; Veit et al., 2007; Wolfe, 2005) and POXB is supplementary (Chang and Cronan, 1983; Chang et al., 1994). However, it has been reported that POXB makes a significant contribution to the aerobic growth efficiency of *E. coli*. In glucose minimal medium, *poxB*-deletion mutant showed ~15% lower growth rate and yield than its parental counterpart. The reason is not clear, but it has been suggested that PoxB may function as a safety valve to convert excess pyruvate to acetate rather than to acetyl-CoA, thus maintaining the intracellular CoA pool for other metabolic functions including the conversion of 2-oxoglutarate to succinyl-CoA and hence citric acid cycle activity (Abdel-Hamid et al., 2001). Under aerobic conditions, the two acetate-forming pathways, PTA-ACKA and POXB, are also affected by pH: acidic environments repress the PTA-ACKA pathway while activating the POXB pathway.

In some microorganisms, including *Pseudomonas* sp., *Klebsiella pneumoniae,* and acetic acid bacteria (AAB), the assimilatory conversion of acetate to acetyl-CoA is carried out by an enzyme known as succinyl-CoA:acetate CoA-transferase (SCACT; EC:2.8.3.18) (**Fig. 2**). SCACT takes CoA from succinyl-CoA and transfers it to acetate while regenerating succinate. Consequently, SCACT can replace PTA-ACKA or ACS in acetate assimilation and, simultaneously, substitute succinyl-CoA synthetase (SCS) in the canonical TCA cycle. SCACT belongs to class I CoA-transferases and exhibits a sequence, structure, and ping-pong kinetic mechanism similar to those of other CoA-transferases (Hersh and Jencks, 1967; Mullins and Kappock, 2012). The ping-pong mechanism involves glutamate as the acceptor residue (Heider, 2001). Recently, Kwong et al. showed that the expression of SCACT in an SCS knockout mutant restored complete operation of the TCA cycle (Kwong et al., 2017). In addition, SCACT could supply acetyl-CoA to the glyoxylate shunt (Kwong et al., 2017; Mullins et al., 2008). Furthermore, *E. coli* strains lacking both

pta-ackA and *acs* that do not grow on acetate as the sole carbon source could be rescued by the heterologous expression of SCACT. In AAB such as *Acetobacter aceti*, the *aarC* gene encoding SCACT appears in the *aarABC* operon along with *aarA* (encoding an NADH-dependent citrate synthase) and *sixA* (encoding a phosphoprotein phosphatase; formerly designated as *aarB*). AAB show acetic acid resistance that is considered a consequence of the presence of the *aarABC* operon (see **3.3 Acetic acid bacteria** for details). This operon is also involved in regulating the gene expression of TCA cycle enzymes in the log growth phase (Mullins and Kappock, 2013, Mullins et al., 2008).

2.2 Glyoxylate shunt

When acetate is the only carbon and energy source, the glyoxylate shunt is required for microbial growth (**Fig. 2**). This shunt starts with the splitting of isocitrate, the second component of the TCA cycle, into glyoxylate (C_2) and succinate (C_4). Glyoxylate then reacts with acetyl-CoA to form malate, a four-carbon dicarboxylic acid. Therefore, the net effect of this bypass is the formation of one molecule of C_4 malate from two molecules of C_2 acetyl-CoA (Dolan & Welch, 2018). Malate is subsequently oxidized to yield fumarate and oxaloacetate, thereby contributing to both the generation of energy and the synthesis of higher carbon compounds through gluconeogenesis. In *E. coli*, the enzymes of the glyoxylate shunt are encoded by the *aceBAK* operon, which is repressed by IclR (*i*so*c*itrate *l*yase *r*egulator).

The first reaction in the glyoxylate shunt is the aldol cleavage of isocitrate to succinate and glyoxylate, catalyzed by isocitrate lyase (ICL or ACEA; encoded by *aceA;* EC 4.1.3.1). ICL, a homotetramer in the active form (Britton et al., 2001; Chao and Liao, 1994; Gainey et al., 1992), requires Mg^{2+} or Mn^{2+} for its activity (Dunn et al., 2009; Hoyt et al., 1991). The analysis of the amino acid sequences of ICL subunits revealed that the molecular weight varied between 48 kDa (prokaryotes) and 67 kDa (eukaryotes). The structures of monomeric ICL as well as the tetrameric complex have been resolved (Anstrom et al., 2003; Britton et al., 2001). Site-directed mutagenesis of *ace A* in *E. coli* revealed that Lys_{193}, Cys_{195}, His_{197}, and His_{356} are critical for the catalytic activity of ICL and that His_{184} is involved in the assembly and phosphorylation of the tetrameric complex (Diehl and McFadden, 1994; Diehl and McFadden, 1993; Rehman and McFadden, 1996; Rehman and McFadden, 1997a; Rehman and McFadden, 1997b; Rehman and McFadden, 1997c). The second reaction in the glyoxylate shunt is the condensation of glyoxylate with acetyl-CoA to malate, catalyzed by malate synthase (MS). MS requires Mg^{2+} for its activity and is competitively inhibited by oxalate, a glyoxylate analog (Dixon et al., 1960; Dolan and Welch, 2018). *E. coli* presents two MSs: malate synthase G (MSG, “G” denotes induction by glycolate; encoded by *glcB*) and malate synthase A (MSA, “A” denotes induction by acetate; encoded by *aceB,* a component of the *aceBAK* operon). MSG is a monomeric enzyme with a molecular mass of ~80 kDa. MSG is present only in bacteria and functions during their growth on glycolate (hydroxyacetate) as the sole carbon source. In contrast, MSA is a multimeric enzyme with a molecular mass of ~ 65 kDa per subunit; it is essential for the growth of plants, fungi, and bacteria on acetate. While structurally similar to MSG, MSA is a little smaller owing to the lack of an α/β domain. The active site of MSA contains Glu_{250} and Asp_{278} for the binding of Mg^{2+} and Pro_{369}, Met_{102}, Thr_{95}, Ala_{367}, Asn_{105}, Lys_{101}, Tyr_{154}, and His_{368} for the binding

of acetyl-CoA ribose. In addition, the active site of MSA contains the highly conserved Cys_{438}, which corresponds to the Cys_{617} in MSG (Lohman et al., 2008).

In the TCA cycle, isocitrate is converted to α-ketoglutarate by the NADP-dependent isocitrate dehydrogenase (ICD; EC 1.1.1.42), which competes with ICL for isocitrate and affects the carbon flux distribution at the isocitrate node. The reaction involved is oxidative decarboxylation with Mn^+ as a cofactor and proceeds in three steps via a ketone group containing oxalosuccinate and an enol intermediate. Typically, ICD has a higher affinity for isocitrate (K_M = 8 μM) than ICL (K_M = 63~604 μM), thus drawing more isocitrate to α-ketoglutarate. The activity of ICD is highly regulated; it is stimulated by relatively high concentrations of ADP and $NAD(P)^+$ and inhibited by ATP and NAD(P)H. Interestingly, ICD activity is also regulated by ACEK (encoded by *aceK,* a component of the *aceBAK* operon), known as ICD kinase/phosphatase (Zheng and Jia, 2010) (See **2.4 Regulation of acetate metabolism** below).

2.3 Transport of acetate

Acetate is transported across the cell membrane by both passive and active transport, with the latter occurring predominantly. During passive transport, the undissociated form of acetic acid (CH_3COOH) from the environment is assumed to enter into the cell via the membrane, through pores in the outer membrane and/or facilitator proteins present in the cell membrane. The passive diffusion of small organic acids is driven by the concentration gradient across the membrane, and the diffusion rate increases when the ratio of free acid to anion is enhanced (Handerson-Hasselbalch eq.) via a reduction in the medium pH. Inside the cell, acetic acid quickly dissociates to an acetate anion and a proton, and the anion usually does not return to the environment. During active transport, acetate is transported by symporters, which are activated by sodium (sodium:solute symporter) or proton (H^+/monocarboxylic acid symporter, PMCT; Transporter Classification Database 2.A.96). The sodium (Na^+):solute symporter (SSS; TCDB 2.A.21) is a large transporter family, often responsible for the absorption of nutrients such as sugars, amino acids, nucleosides, etc. (Reizer et al., 1994; Saier, 2000; Turk and Wright, 1997). According to structural analyses, many SSSs have a characteristic 13-helix arrangement and their configuration is altered by the interaction between Na^+ and the solute binding sites (Jung, 2001). The proton (H^+)/monocarboxylate symporter (TCDB 2.A.96), also known as proton-linked monocarboxylate transporter (PMCT), is common in mammalian cells and tissues. PMCTs are responsible for the transport of monocarboxylates, including pyruvate, lactate, and ketone bodies, and play an essential role in the metabolism of carbohydrates, fats, and amino acids (Enerson and Drewes, 2003; Halestrap and Price, 1999; Halestrap and Wilson, 2012; Morris and Felmlee, 2008). PMCTs do not require energy other than that provided by the concentration gradient of monocarboxylates and protons (Halestrap and Price, 1999; Juel, 1997; Poole and Halestrap, 1993). Sometimes, a clear distinction between SSS and PMCT cannot be made; in *E. coli*, the electrochemical Na^+ gradient can be generated by the action of Na^+/H^+ antiporter(s) (Dimroth, 1997; Jung, 2001).

Acetate permease (ActP), belonging to the SSS family, has been well studied in *E. coli* (Gimenez et al., 2003) and the phototrophic *Rhodobacter capsulatus* (Borghese and Zannoni, 2010). ActP is specific to acetate, glycolate, and propionate. The *actP* gene in *E. coli* (formerly designated as *yjcG*) appears in a polycistronic operon along with *acs* (encoding acetyl-CoA synthetase), and its expression is

elevated during the early stationary phase or upon the depletion of readily usable carbon substrates such as glucose. ActP is different from most other bacterial carboxylate permeases such as GlcA (glycolate permase) and LldP (L-lactate permease), which also recognize two- or three-carbon monocarboxylates. The combined activity of ActP and ACS is assumed to be involved in scavenging excreted acetate from the extracellular medium in *E. coli*. The kinetic properties of ActP for acetate transport have been reported as K_M = 5.4 μM and V_{max} = 19.6 nmol /min/mg protein (Gimenez et al., 2003).

YaaH is a typical PMCT. YaaH family transporters are polytropic proteins with six transmembrane segments containing a conserved motif (N-P-[AV]-P-[LF]-G-L-X-[GSA]-F) located at the first putative membrane region of the N-terminus. The crystal structure of YaaH exhibits a hexameric, UreI-like channel configuration (Sun et al., 2018). In *E. coli*, YaaH is highly specific to acetate and succinate and is thus known as succinate-acetate transporter protein (SATP). The kinetic properties of *E. coli* SATP have been determined: K_M = 1.24 ± 0.13 mM and V_{max} = 8.27 ± 0.37 nmol/min/mg protein for acetic acid (note that the V_{max} of *E. coli* SATP is comparable to that of ActP, but the K_M is ~200-fold higher); K_M = 1.18 ± 0.01 mM and V_{max} = 10.05 nmol/min/mg protein for succinic acid. YaaH family proteins are present in archaea, eukaryotes, and other bacteria, and their functions have been experimentally verified in several microorganisms: Ady2 (accumulation of dyads 2) in *Saccharomyces cerevisiae* (Casal et al., 1996; Paiva et al., 2004), Gpr1 (glyoxylate pathway regulator) in *Yarrowia lipolytica* (Augstein et al., 2003), MA4008 in the archaeon *Methanosarcina acetivorans* (Rohlin and Gunsalus, 2010), and AcpA (acetate permease) and AlcS in the fungus *Aspergillus nidulans* (Robellet et al., 2008). *S. cerevisiae* Ady2 is specific to acetate, propionate, lactate, and formate, and its expression is repressed by glucose (Casal et al., 1996; Pacheco et al., 2012; Paiva et al., 2004). In the gram-positive bacterium *Corynebacterium glutamicum,* MctC (monocarboxylic acid transporter) has been identified as an acetate/proton symporter (Jolkver et al., 2009). Propionate is also transported by MctC, unlike many other monocarboxylates such as pyruvate, *D*(*L*)-lactates, butyrate, succinate, 2-ketobutyrate, *D,L*-hydroxybutyrate, and oxalate. In *C. glutamicum*, passive diffusion was negligible (Ebbighausen et al., 1991; Gerstmeir et al., 2003; Jolkver et al., 2009).

2.4 Regulation of acetate metabolism

Acetate metabolism is regulated in a complex manner at different levels, including transcription, translation, (posttranslational) protein structure, and even protein activity (regulated by small molecules) (Bernal et al., 2016). The global regulators of acetate metabolism include catabolite repressor activators (Cra and/or FruR), cyclic AMP receptor protein (CRP), aerobic respiration control protein (ArcA/B), and CreBC (**Fig. 3**). Protein modifications include phosphorylation and acetylation. All of these controls reflect the cellular demand for optimal growth under different culture conditions (especially with regard to the carbon source and availability of oxygen) and involve glycolysis, gluconeogenesis, the pyruvate dehydrogenase complex, the TCA cycle, and the glyoxylate shunt (Li et al., 2014; Perrenoud and Sauer, 2005; Shalel-Levanon et al., 2005). An excellent review paper covering the details for *E. coli* has already been published (Bernal et al., 2016); therefore, here, this part is described briefly.

During *E. coli* cultivation on glucose, *pta* and *ackA* are upregulated by 1.3- and 1.7-fold, respectively, under microaerobic conditions. In contrast, on acetate, their expression and the enzymatic activities of PTA and ACKA are reduced by 30~50% compared to those observed during aerobic growth on glucose. As a consequence, it has been proposed that PTA-ACKA is mainly responsible for acetate formation (and excretion) under fermentative conditions, whereas ACS is responsible for acetate assimilation (Oh et al., 2002). The expression of *pta-ackA* is directly controlled by at least two global transcriptional regulators, FNR (*f*umarate and *n*itrate *r*eductase) and CreBC and indirectly by ArcA (Bernal et al., 2016; Li et al., 2014; Shalel-Levanon et al., 2005). FNR is an oxygen-sensing global regulator and an iron sulfur-dependent DNA-binding protein. FNR, serving as the transcriptional activator of many anaerobically regulated genes, increases the expression of *pta* and *ackA* (thus, acetate formation) during growth on glucose (Gunsalus and Park, 1994; Marzan et al., 2013). Similarly, CreBC upregulates the transcription of the *pta-ackA* operon. CreBC is encoded by the four-gene cluster, *creABCD* (Amemura et al., 1986), where CreC is a membrane-associated protein kinase that phosphorylates the response element CreB (Amemura et al., 1990; Yamamoto et al., 2005). The CreBC system is activated when glycolytic carbon sources are fermented (Avison et al., 2001; Cariss et al., 2008; Caspi et al., 2014; Godoy et al., 2016; Kakuda et al., 1994; Sprenger, 1995). Moreover, regulation by CreBC is affected by oxygen availability (Bernal et al., 2016; Godoy et al., 2016). In comparison to FNR or CreBC, ArcAB mainly regulates TCA cycle genes depending on the oxygen level or redox state. *arcA* deletion in *E. coli* activates the TCA cycle and reduces acetate formation (Vemuri and Aristidou, 2005; Vemuri et al., 2005). Therefore, the decrease in acetate production following *arcA* deletion is rather indirect, most likely due to the acceleration of the TCA cycle under oxygen-limited conditions. However, in cells growing on acetate as the carbon source (which should be aerobic), the importance of FNR, CreBC, and/or ArcAB for the transcription of the *pta-ackA* operon remains elusive.

The activity of ACS is upregulated at the transcriptional level by CRP, the major regulatory protein for carbon catabolite repression. In contrast, ACS activity is inhibited by acetylation at the posttranslational level, which is relieved by deacetylation. In *E. coli* K12, PatZ, a Gnc5-like acetyltransferase, functions as an acetylase (de Diego Puente et al., 2015), whereas CobB functions as an NAD^+-dependent protein deacetylase (also known as “sirtuin”) (Castano-Cerezo et al., 2011; Castano-Cerezo et al., 2014; de Diego Puente et al., 2015; Weinert et al., 2013). At high acetyl-CoA levels, PatZ is activated by autoacetylation and subsequently oligomerizes to an octamer (de Diego Puente et al., 2015). Therefore, the inactivation of ACS by acetylation is an “indirect” product inhibition that is mediated by peptidyl-lysine *N*-acetyltransferase PatZ. Interestingly, the expression of both *patZ* and *acs* is upregulated by cAMP-CRP when glucose is absent (Bernal et al., 2016; Castano-Cerezo et al., 2011; Hentchel et al., 2015; Kumari et al., 2000). This indicates that both ACS and PatZ, functioning in two opposite directions, are upregulated concurrently. It has been suggested that acetylation acts as a fine-tuning mechanism of ACS activity, i.e., acetylation by PatZ prevents ACS activity from reaching extreme levels when *acs* expression is induced. Fine-tuning of ACS activity is important for maintaining cellular homeostasis for acetyl-CoA and ATP. The pool size of acetylating agents (i.e., acetyl-CoA and acetyl-P) is greatly affected by ACS activity and should not be too large to avoid the extensive acetylation of cellular

proteins. Furthermore, excessive ACS activity causes unbalanced ATP usage and the extensive accumulation of AMP. In *patZ*-deficient *Salmonella enterica* strains, the overexpression of ACS was deleterious during growth on acetate (Avison et al., 2001; Gardner et al., 2006).

In *E. coli* and other *Enterobacter* spp., glyoxylate shunt enzymes encoded by the *aceBAK* operon are negatively regulated by IclR (ICL regulator). During growth on glucose, the glyoxylate shunt is largely suppressed. In contrast, in cells growing on acetate or fatty acids, IclR repression is relieved and the transcription of the *aceBAK* operon is enhanced. Several metabolites such as glyoxylate, pyruvate, and phosphoenolpyruvate (PEP) are involved in this regulation as effectors (Cortay et al., 1991; Cozzone, 1998; Gui et al., 1996a; Gui et al., 1996b; Lorca et al., 2007). For example, pyruvate increases the binding of IclR to the *aceBAK* operon (Cortay et al., 1991). The expression of IclR is repressed by itself in a ligand-independent manner. FadR, a transcriptional regulator involved in fatty acid metabolism, is also known to positively regulate the transcription of *iclR*. The regulatory pair FadR/IclR coordinates the breakdown of fatty acids and the utilization of acetyl-CoA via the glyoxylate shunt (Gui et al., 1996a; Gui et al., 1996b).

ICD (isocitrate dehydrogenase) activity is important in controlling the flux distribution at the isocitrate node. The activity of ICD is stimulated by relatively high concentrations of ADP and $NAD(P)^+$ and inhibited by ATP and NAD(P)H. In addition, the activity of ICD is regulated at the protein level by ACEK, which is encoded from the *aceBAK* operon of the glyoxylate shunt. ACEK bifunctionally catalyzes the reversible phosphorylation (as kinase) and dephosphorylation (phosphatase) of ICD at Ser_{113} on the active site. ICD is inactivated when phosphorylated (by the kinase activity of ACEK) and the carbon flux toward the glyoxylate shunt is increased (Cozzone, 1998; Dolan and Welch, 2018; Garnak and Reeves, 1979a; Garnak and Reeves, 1979b; LaPorte and Koshland, 1982). In contrast, when dephosphorylated (by the phosphatase activity of ACEK), ICD is activated and the carbon flux toward the TCA cycle is increased. The introduction of the negatively charged phosphate group into the active site of ICD (Ser_{113}) results in electrostatic repulsion to the native isocitrate substrate, thus inactivating the enzyme. During growth on acetate, about 75% of ICD is inactivated (Clark and Cronan, 1996; Cozzone et al., 1998). ACEK regulation to function as either kinase or phosphatase has been studied *in vitro,* but the fine mechanisms underlying this process remain unclear. ACEK possesses two functional domains: a catalytic domain that performs kinase, phosphatase, and ATPase functions and a regulatory domain that contains allosteric binding pockets for the regulation of the catalytic domain (Zheng and Jia, 2010). Several metabolites, including AMP, ADP, 3-phosphoglycerate, α-ketoglutarate, and pyruvate, have been shown to stimulate phosphatase activity by binding to the regulatory domain of ACEK and concomitantly inhibiting kinase activity. Isocitrate and NADPH inhibit ACEK kinase (ACEK-K) activity; NADPH also inhibits ACEK phosphatase (ACEK-P) activity. However, these effectors bind to ICD rather than ACEK kinase/phosphatase; the binding of these ligands in ICD prevents the formation of the ACEK kinase/phosphatase complex (Miller et al., 2000; Zheng and Jia, 2010). Interestingly, ATP is a phosphate donor for kinase activity but also acts as an activator of ACEK phosphatase (Miller et al., 2000; Nimmo and Nimmo, 1984; Zheng and Jia, 2010).

Acetylation and deacetylation mediated by PatZ and CobB, respectively, are also involved in controlling the activity of ICL, ACEK, and a glycolytic enzyme, glyceraldehyde-3-phosphate dehydrogenase (Wang et al., 2010). In *S. enterica*, a *cobB*-deficient mutant grew faster than the wild-type on glucose but slower in the presence of citrate as the sole carbon source (Wang et al., 2010). Moreover, ^{13}C-flux analyses revealed that a *patZ*-deficient mutant exhibited increased flux through the glyoxylate shunt. The slower growth of *cobB*-deficient mutants on citrate or acetate suggests that the glyoxylate shunt operates less efficiently without *cobB* (Castaño-Cerezo et al., 2014). At the protein level, ICL or ACEK becomes acetylated (owing to the acetylase activity of PatZ), which reduces their enzymatic activity. The decreased ACEK activity leads to an increase in the activity of ICD by reducing its phosphorylation and reduces the flux toward the glyoxylate bypass. However, in another study, these effects of ICL and ACEK acetylation could not be reproduced *in vitro* (Crosby et al., 2012). Acetylation appears to mediate more of a modulatory response and may fine-tune fluxes in response to subtle changes in nutrient availability (Dolan and Welch, 2018).

The regulation of acetate metabolism has also been studied in the gram-positive bacterium *C. glutamicum* (Holms, 1996; Walsh and Koshland, 1985; Wendisch et al., 2000). In *C. glutamicum*, ICL and MS are subject to allosteric regulation by metabolic intermediates of central metabolism (Reinscheid et al., 1994a; Reinscheid et al., 1994b). Notably, unlike *E. coli*, *C. glutamicum* does not have a functional homolog of IclR. Instead, a protein known as RamB (*r*egulator of *a*cetate *m*etabolism) functions as a transcriptional regulator of glyoxylate shunt genes. The 53-kDa protein binds to the intergenic region between *aceA* and *aceB* as well as the promoter/operator region of the *pta-ackA* operon and represses the transcription of these three genes in the absence of acetate (Gerstmeir et al., 2004; Gerstmeir et al., 2003). The 13-bp DNA motif (CAAAATTTGCAAA) for binding RamB is highly conserved in other microorganisms such as *Mycobacterium tuberculosis.* Apart from RamB, there were several common regulatory features as seen in *E. coli*: (i) inhibition of the glyoxylate shunt by glycolytic intermediates and succinate, (ii) inhibition of the TCA cycle by oxaloacetate and glyoxylate at the level of ICD, and (iii) inhibition of the TCA cycle by high levels of ATP at citrate synthase and gluconeogenesis. Similar to *E. coli*, flux distribution at the isocitrate branching point between the TCA cycle and glyoxylate shunt is additionally governed by the intracellular concentration of isocitrate through the different affinities of ICL and ICD toward their common substrate (280 and 12 mM, respectively) (Gerstmeir et al., 2003).

3. MICROBIAL GROWTH ON ACETATE

Microbial growth on acetate occurs mainly under aerobic conditions, when energy production is possible by the catabolism of acetate through the TCA cycle and electron transport chain (ETC). For anaerobic growth, other carbon sources capable of supplying the reducing power and ATP and/or alternative electron acceptors that can oxidize acetate under anaerobic conditions are needed. For either aerobic or anaerobic growth, gluconeogenesis is essential for the synthesis of higher carbon compounds from C_2 acetate. In this chapter, the growth characteristics of microorganisms on acetate are described, particularly in comparison with glucose. Global changes in gene expression, the adaptive evolution of cell growth on acetate, and the toxic effects of acetate are also summarized.

3.1 Growth characteristics on acetate

Acetate assimilation takes place primarily through the glyoxylate shunt and less frequently via the ethylmalonyl-CoA pathway and methylaspartate cycle (Borjian et al., 2016; Dolan and Welch, 2018; Ensign, 2011; Qiao et al., 2014; Schneider et al., 2012). The ethylmalonyl-CoA pathway is present in α-proteobacteria, like *Rhodobacter sphaeroides* and *Methylobacterium extorquens*, as well as actinomycetes, like *Streptomyces* spp (Alber, 2011; Laguna et al., 2011; Schneider et al., 2012), whereas the methylaspartate cycle has been reported in the halophilic *Haloarcula marismortui* and *H. hispanica* (Ensign, 2011; Borjian et al., 2016). All three pathways are essential for cell growth when C_1 or C_2 compounds are used as sole carbon and energy source, because they are needed to convert acetyl-CoA, a 2-carbon compound, to a 4-carbon compound that could feed anaplerotic reactions, i.e. the reactions that form metabolic intermediates for biosynthesis. The glyoxylate shunt, the simplest among the three, is a modified version of the TCA cycle that bypasses the steps in the TCA cycle that lead to a loss of CO_2. Acetyl-CoA enters the cycle at two steps, and the output is the 4-carbon compound succinate (see **2.2 Glyoxylate shunt**). In the ethylmalonyl-CoA pathway, a C_4-compound, acetoacetyl-CoA, derived from two acetyl-CoA molecules, is converted to the C_5-compound 2-methylfumaryl-CoA. (2*R*,3*S*)-β-methylmalyl-CoA, formed by hydration of 2-methylfumaryl-CoA, is cleaved to glyoxylate and propanoyl-CoA. Condensation of glyoxylate and another molecule of acetyl-CoA yields (*S*)-malate, while propionyl-CoA is carboxylated to succinate, a TCA intermediate, via (2*S*)-methylmalonyl-CoA, (2*R*)-methylmalonyl-CoA and scuccinyl-CoA. Collectively, in this cycle, two molecules of glyoxylate, the intermediate of serine cycle, are formed from one molecule of acetate, one molecule of CO_2 and one molecule HCO_3^-. It should be noted that the ethylmalonyl-CoA pathway is integrated with two cycles, the serine cycle and TCA cycle, both of which are essential for cell growth. In the third, methylaspartate cycle, acetyl-CoA is transformed to glutamate via reactions of the TCA cycle and glutamate dehydrogenase. The rearrangement of glutamate into methylaspartate and its following deamination leads to mesaconate, and mesaconate is activated to mesaconyl-CoA and hydrated to β-methylmalyl-CoA. The β-methylmalyl-CoA is finally cleaved to propionyl-CoA and glyoxylate. Propionyl-CoA carboxylation leads to methylmalonyl-CoA and subsequently to succinyl-CoA, thus closing the cycle, whereas the condensation of glyoxylate with another molecule of acetyl-CoA yields the final product of the methylaspartate cycle, malate. In total, two molecules of acetyl-CoA and one molecule of oxaloacetate are transformed to succinyl-CoA and malate (Borjian et al., 2016). Interestingly, Halobacteria containing the methylaspartate cycle also have glyoxylate shunt, and the former cycle is known to be used during starvation in the process of converting poly-β-hydroxybutyrate, which is largely accumulated during bloom conditions, to biosynthetic intermediates. Further, because the affinity of methylaspartate ammonia-lyase for methylaspartate is very low, a functioning methylaspartate cycle is expected to require high intracellular glutamate concentration. In fact, cytoplasmic glutamate concentration in acetate-grown *H. marismortui* cells is known to be very high as ~35 mM. However, this is not unexpected, because glutamate is a well-known osmolyte used by many Halobacteria (Borjian et al., 2016).

The intentional use of acetate as the major carbon feedstock for microbial growth and the production of metabolites started only recently. The growth rate varies

between microbe species and depends particularly on the addition of yeast extract to the culture medium (**Table 2**). However, the direct comparison of kinetic data reported by different research groups is difficult owing to the different culture conditions and obscure estimation methods. On acetate, the gram-negative bacterium *Thiobacillus* showed a specific growth rate of 0.22 h^{-1} (Gottschal and Kuenen, 1980), whereas another gram-negative bacterium *Azotobacter vinelandii* showed a specific growth rate of 0.35 h^{-1} (Tauchert et al., 1990). The growth of *E. coli* has been studied extensively; the specific growth rate was 0.20 ~ 0.25 h^{-1} without yeast extract and 0.40~0.55 h^{-1} with yeast extract supplementation. Rajaraman et al. (2016) compared 18 different *E. coli* strains and reported that their specific growth rates varied between 0.15 and 0.41 h^{-1} when grown on minimal medium containing 1.7 g/L citric acid and 6.8 g/L acetate (Rajaraman et al., 2016). They suggested that the differences in the growth rates could be attributed to the variation in acid tolerance (Rajaraman et al., 2016). Ibarra et al. (2002) adapted *E. coli* K12 MG1655 to acetate on yeast extract-free minimal medium and achieved a specific growth rate of 0.25 h^{-1} on 2 g/L acetate (Ibarra et al., 2002)(Ibarra et al., 2002). Noh et al. (2018) compared four different *E. coli* strains—W, BL21(DE3), W3110, and MG1655—on minimal medium containing 2 g/L yeast extract and 10 g/L acetate (Noh et al., 2018). The strain W showed the highest specific growth rate (0.46 h^{-1}) and the highest acetate consumption rate (0.22 g/g DCW/h). Similarly, we also compared six different *E. coli* strains—B, BL21, C, K12, W, and W3110—on minimal medium containing 1 g/L yeast extract and 4 g/L acetate (unpublished). The specific growth rate varied between 0.41 and 0.57 h^{-1}, and the acetate consumption rate varied between 0.82 and 0.94 g/g DCW/h. When yeast extract was not included in the culture medium, the specific growth rate was reduced to ~0.20 h^{-1}. Yang et al. (2019) evaluated three different *Pseudomonas* strains—*P. putida* KT2440, *P. putida* NBRC14164, and *P. aeruginosa* PH1—on minimal medium containing 10 g/L acetate (Yang et al., 2019) . *P. putida* KT2440 showed the highest specific growth rate (0.21 h^{-1}) and acetate consumption rate (0.20 g/g DCW/h), whereas *P. putida* NBRC14164 was not able to grow at all on acetate. The growth and acetate consumption rate of *P. aeruginosa* PH1 were 0.18 h^{-1} and 0.125 g/g DCW/h, respectively. *C. glutamicum*, a gram-positive bacterium, had a specific growth rate of 0.28 h^{-1}, acetate consumption rate of 540 nmol/mg protein/min (equivalent to ~0.97 g/g DCW/h), and growth yield of 0.29 g/g acetate (Wendisch et al., 2000).

The acetate concentration in the culture medium greatly affected the specific growth rate. Generally, the specific growth rate increases or remains constant up to a threshold level, above which toxic effects appear and the growth rate decreases in a dose-dependent manner (Schneider et al., 2012). In *C. glutamicum*, the specific growth rate was 0.32 h^{-1} at 60 mM acetate (3.6 g/L) and decreased to 0.24 h^{-1} at acetate concentrations above 180 mM (10.8 g/L) (Wendisch et al., 2000). Similarly, *Methylobacterium extorquens* AM1 had a specific growth rate of 0.068 h^{-1} at 5 mM acetate (0.3 g/L) and 0.025 h^{-1} at 30 mM (1.8 g/L) (Schneider et al., 2012). Xiao et al., (2013) cultured *E. coli* BL21(DE3) at acetate concentrations ranging between 25 and 100 mM (1.5 ~ 6 g/L) and obtained the highest cell biomass at 50 mM and the lowest biomass at 100 mM (Xiao et al., 2013).

3.2 Gene expression profiles in acetate-growing cells

Global gene expression was investigated to understand cellular responses to acetate and gain insight for improving tolerance to acetate (also see **3.4. Toxicity of acetate and tolerance mechanism**). In general, in cells grown on acetate or exposed to acetate, acetate metabolic genes (glyoxylate shunt, gluconeogenesis and TCA cycle) are upregulated, whereas genes involved in sugar metabolisms (glycolysis, pentose phosphate pathway, and sugar transport systems), cell replication and the transcription-translation machineries are downregulated. Arnold et al. (2001) reported that the expression of a total of 86 genes was altered in *E. coli* after exposure to acetic acid during growth on glucose. About 30% were upregulated, including *gadABC*, *cfa*, *hdeAB*, *katE*, *dps*, and *grxB*, known to provide protection against acetic acid through amino acid decarboxylation, cyclopropane fatty acid synthesis, and chaperones. In contrast, the remaining 70% (~60 genes) were downregulated, including genes involved in the transcription and translation machineries, such as L10, *rplMU, rpmB, rpoA, rpsABOP, spc, str,* and *S10,* and some other metabolic pathways, such as the *leu* operon, *cysK*, *ptsG*, *mipA*, *ompF*, *prlA*, *ybiK*, *yceD,* and *yeeEF*. Generally, the repression levels were less significant than the induction levels. Oh et al. (2002) compared the gene expression profiles of *E. coli* after shifting the carbon source from glucose to acetate (Oh et al., 2002) On acetate, genes involved in acetate intake (*acs*), glyoxylate (*aceABK*), and gluconeogenesis (*pckA*, *ppsA*) were significantly enhanced (8–21-fold). To a lesser extent, several other genes in gluconeogenesis (*sfcA* and *maeB*) and TCA were also upregulated. In contrast, genes of the Embden-Meyerhof-Parnas (EMP) pathway (*pfkA, fba, gapA, epd, pgk, eno, pykF,* and *ppc*) and glucose transport system (*ptsHI-crr* operon and *ptsG*) were downregulated (up to four fold). In addition, the first two genes of the pentose phosphate pathway (*zwf* and *gnd*) and pyruvate dehydrogenase (*aceEF* operon) were significantly downregulated. Peng and Shimizu (2003) studied the gene expression profiles of *E. coli* growing on several carbon sources, including acetate, glucose, glycerol, and gluconate (Peng and Shimizu, 2003), and obtained similar results as Oh et al. (2002) and Arnold et al. (2001). On acetate, the EMP and pentose phosphate pathways (*pfkA*, *pykF*, *ppc,* and *zwf*) were downregulated, whereas gluconeogenesis (*fbp*, *pckA*, *ppsA,* and *mez*) and the glyoxylate cycle (*aceABK*) were significantly upregulated (>10-fold).

In addition to *E. coli*, global gene expression profiles during the growth on acetate were also studied in *C. glutamicum* (Gerstmeir et al., 2003; Hayashi et al., 2002), *Acetobacter aceti* (Sakurai et al., 2011) and *S. cerevisiae* (Daran-Lapujade et al., 2004). In *C. glutamicum*, when grown on acetate, ~ 60 genes involved in the acetate assimilation (*pta-ackA*), glycoxylate shunt (*aceA* and *aceB*), TCA cycle (*gltA*, *acn*, *fum*, and *sdhABCD*), and gluconeogenesis (*pck*) were significantly upregulated (2–28 fold). On the other hand, the genes involved in the phosphotransferase sugar transport system (*ptsM* and *malP*), pentose phosphate pathway (*zwf2*, *tal*, and *tkt*), glycolysis (*aceE*), and malic enzyme (*malE*) were down-regulated (1.8–4.2 fold). Generally, the change of gene expression profiles induced by acetate was very similar between *C. glutamicum* and *E. coli* except the *pta-ackA* operon; the *pta-ackA* operon was upregulated in *C. glutamicum* (3.1–6.2 fold) (Gerstmeir et al., 2003), but downregulated in *E. coli* (2-fold) (Oh *et al.*, 2002). The transcriptomic analysis of acetic acid bacteria *Acetobacter aceti* NBRC 14818 was also conducted for the cells growing on three carbon sources such as acetate, ethanol, and glucose (Sakurai *et al.*, 2011; **see 3.3 Acetic acid bacteria**). Genes for the TCA cycle (*aarA*, *acnA*, *icd1*,

icd2, *sucAB*, *sucCD*, *sdhABCD*, *fumA*, *fumC*, and *mqo*), glyoxylate shunt (*aceA* and *glcB*), two putative acetyl-CoA synthetase (*acs1* and *acs2*), EMP pathway (*fabB* and *glpX*), and alcohol dehydrogenases (pyrroloquinoline quinone-dependent and NAD^+-dependent) were significantly upregulated in response to acetate. Especially, expression of *acnA* and *aarC* were greatly upregulated in the acetate-grown cells in comparison to the ethanol- or glucose-grown cells (1.3–6.5 fold). For *S. cerevisiae*, genome-wide transcription profiles have also been reported for the cells grown on different carbon sources such as C_6 (glucose or maltose) and C_2 (ethanol or acetate) compounds (Daran-Lapujade *et al.*, 2004). When grown on C_2 compounds, most of the C_2-responsive genes (117 of 180 genes) were differentially expressed. Upregulated genes included the ones related to the gluconeogenesis and glyoxylate cycle (*pck1*, *fbp1*, *icl1*, icl2, and *mls1*), TCA cycle (*cit2*, *idh1*, *idh2*, *fum1*, *sdh1*, *sdh3*, and *mdh2*), acetyl-CoA metabolism and its trafficking across the membranes (*acs1*, *ach1*, *cat1*, *yat1*, and *yat2*), and transport across the cytosolic and the mitochondrial membranes (*crc1*, *sfc1*, and *stl1*). Of the 38 genes downregulated in the presence of acetate, genes linked to the glycolysis (*hxk1* and *tdh1*), pentose phosphate pathway (*tkl2*, *gnd1*, and *gnd2*), members of the hexose transport family *(hxt2* and *hxt7*), and iso-2-cytochrome C (*cyc7*) involved in respiration were included.

3.3 Acetic acid bacteria

Acetic acid bacteria (AAB) are used in the commercial production of vinegar. They occupy a unique niche in acetic acid metabolism owing to their prominent tolerance to acetic acid as they can grow in the presence of ~100 g/L acetate. Acetic acid bacteria are gram-negative obligate aerobic bacteria, belonging to α-Proteobacteria. At present, 17 genera consisting of 88 species are recognized, with many belonging to *Acetobacter*, *Gluconobacter*, *Gluconacetobacter*, *Asaia*, and *Komagataeibacter* (Matsushita et al., 2016). AAB can produce acetic acid from ethanol through oxidative fermentation. Ethanol is first oxidized to acetaldehyde by a membrane-bound pyrroloquinoline quinone (PQQ)-dependent alcohol dehydrogenase (ADH); subsequently, acetaldehyde is oxidized to acetic acid by aldehyde dehydrogenase (ALDH), which is also membrane-bound (Chinnawirotpisan et al., 2003). The produced acetic acid can be further oxidized to carbon dioxide and water (Saichana et al., 2015). Although AAB are tolerant to acetic acid, their tolerance level varies substantially between species; *Acetobacter* spp. and *Komagataeibacter* spp. are more resistant (up to 100 g/L) than others (Gullo et al., 2014). They are also tolerant to ethanol (up to 94 g/L). The strains produce polysaccharides that help them float on the surface of the medium, supporting the uptake of oxygen and protecting against various stresses such as high acidity and high temperature.

In AAB, acetic acid is incorporated through a specialized TCA cycle, in which SCS and malate dehydrogenase are substituted by succinyl-CoA:acetate CoA-transferase (SCACT; see **2. Metabolism of acetate in microorganisms; Fig. 2 and 3**) and malate:quinone oxidoreductase, respectively. Fukaya et al. (1990) demonstrated that the absence of *aarA* (encoding citrate synthase), *aarB* (encoding a TCA cycle regulator), and/or *aarC* (encoding succinyl-CoA:acetate CoA-transferase) in *Acetobacter aceti* dramatically decreases the tolerance to acetate (Fukaya et al., 1990). The role of AarC in acetic acid assimilation and tolerance was further explored in several other studies (Fukaya et al., 1993; Mullins et al., 2008).

The expression of another TCA cycle enzyme, aconitase, was also enhanced in response to acetic acid present in the culture medium. *A. aceti* harboring multiple copies of the aconitase-encoding gene (*acnA*) showed higher acetic acid tolerance, probably by strengthening the TCA cycle throughput (Nakano and Fukaya, 2008; Nakano et al., 2004). These results collectively suggested that the activation of enzymes involved in acetic acid assimilation confers acetic acid resistance to host strains. In addition to stimulating the TCA cycle, PQQ-dependent ADH was also found to be important in acetic acid tolerance; the strain devoid of this membrane-bound ADH exhibited significantly reduced tolerance to acetic acid (Chinnawirotpisan et al., 2003; Trcek et al., 2006).

3.4 Toxicity of acetate and tolerance mechanism

Acetic acid is known to be toxic to microbial cells, often at concentrations lower than 5 g/L. The toxic effect in the cell occurs globally; thus, the exact mechanism of toxicity remains controversial. Acetic acid is known to be toxic to microbial cells, often at concentrations lower than 5 g/L. The toxic effect in the cell occurs globally; thus, the exact mechanism of toxicity remains controversial. Generally, toxic effects of short-chain aliphatic acids including acetate can be broadly divided into two categories, ‘general’ and ‘anion-specific’ (**Fig. 4A**). Damage to membrane integrity due to accumulation of the acids in the cell membrane, decrease in cellular pH, perturbation of membrane potential essential for oxidative ATP synthesis, anion imbalance, etc. can be considered as ‘general’ toxic effects, whereas inhibition on the activity of specific enzymes and/or pathways, metabolic perturbation, and/or generation of toxic metabolites from the acid, all of which are caused by the nature of a specific anion, can be classified as ‘anion-specific’ toxic effects (Axe and Bailey, 1995; Kildegaard et al., 2014). Structurally, acetic acid is small and hydrophilic; thus its accumulation in the cellular membrane and the subsequent probable damage to membrane integrity are not high. On the other hand, due to the small size, acetate can pass through cell membrane rather easily by simple diffusion and the toxic effects occurring in the cytoplasm, by both proton and anion, can be higher than other organic acids of bigger size. It is known that the passive diffusion undergoes with only undissociated or free form of an acid, the concentration of which in the culture medium is determined by pK_a value and medium pH (i.e., by Henderson-Hasselbalch equation) (see **2.3 Transport of acetate**). With acetate, the effect of pH on its toxic effects, in relation with the free-acid concentration, has not been studied. However, toxic effect of two three-carbon compounds, 3-hydroxypropionic acid (3-HP) (pK_a = 4.50) and its structural isomer lactic acid (pK_a = 3.86), has been studied at varying pH. Interestingly, when determined by growth inhibition to *E. coli*, 3-HP was much more toxic than lactic acid at the same acid concentration in the culture medium. Further, the toxicity of 3-HP and lactic acid becomes similar when the estimated free-acid concentrations in the culture medium were adjusted to be the same (Chun et al., 2014). These results suggest that toxic effect of small organic acids, capable of entering cells by simple diffusion, are highly dependent on their free-acid concentration in the culture medium. As to anion-specific toxic effect, acetate has been reported to cause the depletion of the glutamate pool and the inhibition of methionine biosynthesis (Roe et al., 1998; Roe et al., 2002). However, it is not clear whether this is limited to acetate anion, i.e., truly acetate-anion-specific or not. Acetate can affect acetylation of many enzymes globally and homeostasis of

CoA pool, but this has not been studied in the context of toxic effect (see **2.4 Regulation of acetate metabolism**).

Bacteria have evolved various defense mechanisms, some of which are specific to acetic acid, whereas others are more general to many similar acids (**Fig. 4B**). These mechanisms can be classified into five groups: (i) rapid acetate catabolism, (ii) export of acetate, (iii) amino acid decarboxylation/protonation to mitigate intracellular acidification, (iv) change in cell morphology and membrane composition to limit acetate uptake, and (v) expression of stress-response proteins. The first mechanism has been well established in acetic acid bacteria (AAB) AAB as described before. The second mechanism has also been well studied in AAB. Two types of active transporters—the proton motive force-dependent transporter (H^+/acetic acid antiporter) and the ATP-dependent ABC transporter—have been reported in *A. aceti* (Nakano and Fukaya, 2008). The existence of proton motive force-dependent transporters was illustrated based on the observation that acetic acid in the intact cells and membrane vesicles of *A. aceti* decreased in the presence of respiratory substrates (lactate or succinate) (Matsushita et al., 2005). The ATP-dependent ABC transporter AatA, comprising 591 amino acids and belonging to the macrolide antibiotic efflux pump, was studied (Nakano et al., 2006). The protein had a conserved sequence, characteristic of the ABC transporter superfamily, i.e., ABC signatures I and II and Walker A and B motifs. The deletion of *aatA* in *A. aceti* conferred reduced resistance to acetic acid, whereas its expression recovered this tolerance. Furthermore, *aatA*, when properly expressed, improved the acetic acid tolerance of recombinant *E. coli*.

Another well-known bacterial mechanism of resistance to acetic acid is the amino acid decarboxylation system. This mechanism protects cells not only from acetic acid but also from other organic acids. Four different systems have been reported so far, each one depending on glutamate (Gad), arginine, lysine, or glutamine (Gong et al., 2003; Lu et al., 2013; Richard and Foster, 2004; Rollan et al., 2003; Teixeira et al., 2014). The best-known Gad system utilizes the decarboxylation of glutamate to gamma-aminobutyric acid (GABA) with the consumption of one proton by pyridoxal-dependent glutamate decarboxylase, encoded by *gadA* or *gadB*. The produced GABA is exchanged with extracellular glutamate through the antiporter GadC, encoded by *gadC* (Richard and Foster, 2004; Teixeira et al., 2014). Thus, the net effect is the removal of a cytoplasmic proton at the expense of one glutamate molecule. The three-step arginine deiminase (ADI) pathway is another mechanism for bacterial resistance to acetic acid. In this pathway, arginine is converted to carbon dioxide and ammonia in an enzymatic reaction catalyzed by ADI, ornithine transcarbamoylase, and carbamate kinase. The produced ammonia, an alkaline substance, increases the intracellular pH and promotes cell survival under acidic conditions (Rollan et al., 2003; Teixeira et al., 2014). Furthermore, ornithine, the intermediate produced in this pathway, also contributes to the repair of the DNA damage caused by acid stress.

Another important mechanism for resisting high concentrations of acetic acid is the alteration of cell morphology and membrane composition. Deeraksa et al. (2005) showed that *A. tropicalis* survives at high concentrations of acetic acid by forming pellicle polysaccharides around the cell (Deeraksa et al., 2005). Similarly, Kanchanarach et al. (2010) observed that pellicle polysaccharides formed at the late-

log and stationary phases conferred *A. pasteurianus* cells with resistance to acetic acid during acetic acid fermentation (Kanchanarach et al., 2010). Trček et al. (2007) observed a change in the total fatty acid composition of the membrane when *Gluconacetobacter europaeus* was exposed to acetic acid (Trček et al., 2007). The major unsaturated fatty acids, particularly phosphatidylglycerol, increased by 7.3-fold, whereas phosphatidylethanolamine decreased by 2.7-fold in the presence of 3% (v/v) acetic acid. Similarly, *E. coli* O157:H7 increases the ratio of *cis*-vaccenic to palmitic acid during adaptation to acetic acid (Yuk and Marshall, 2005). The formation of cyclopropane fatty acid (CFA) is a post-synthetic modification of the lipid bilayer, which occurs in *E. coli* when it enters the stationary phase. There is a positive correlation between CFA levels and acetic acid resistance; *E. coli* lacking CFA were highly sensitive to acidic conditions and resistance was recovered by the restoration of CFA (Chang and Cronan, 1999). In *A. aceti*, phosphatidylcholine, one of the major phospholipids, was found to be important in acetic acid tolerance; mutants lacking phosphatidylcholine biosynthesis showed slower growth rate and lower biomass yield on acetate (Hanada et al., 2001). Heipierper et al. (1994) also suggested that the isomerization of *cis*- to *trans*-unsaturated fatty acids may also contribute to acetic acid tolerance in *P. putida* during growth on acetate (Heipieper and de Bont, 1994).

Finally, the genes involved in the stress response and protein folding (*groES*, *groEL*, *dnaK*, *dnaJ,* and *grpE*) have also been suggested to play an important role in acid tolerance (Andres-Barrao et al., 2012). These chaperone proteins protect other proteins by preventing misfolding or promoting refolding and the proper assembly of denatured proteins. However, the detailed mechanism has not yet been fully elucidated. *A. aceti* overexpressing the *groESL* operon was more resistant to acetic acid than the control strain (Nakano and Fukaya, 2008).

Generally, the aforementioned five defense mechanisms are found differently among microorganisms and/or depending on culture conditions. Further, operation of each mechanism causes inherent problems with respect to acetate metabolism and/or product formation to be synthesized from acetate. For example, amino acid decarboxylation system is energetically highly expensive because the removal of one cytoplasmic proton should sacrifice one molecule of amino acid such as glutamate, arginine, lysine, or glutamine (category iii). In the same context, overexpression of chaperon proteins and their functioning (category v) diverts valuable cellular resources of amino acids and energies. Similarly, export of acetate (category ii) or change in cell morphology and membrane composition to limit acetate uptake (category iv) reduces incorporation of the substrate acetate, thus can interfere with the rapid use of acetate when target product is synthesized. It is possible to increase tolerance against acetate by adaptive laboratory evolution (ALE) and some examples have already been reported (see **3.5 Improvement of cell growth on acetate).** Also, the tolerable strains have been analyzed to help the understanding of the relationship between the genotype and phenotype. However, due to the problems associated with energy consumption and/or acetate metabolisms, actual use of the artificially-developed acetate-tolerant strains for biotechnological purposes with acetate as feedstock is limited.

3.5 Improvement of cell growth on acetate

For biotechnological applications, several groups have tried to enhance cell growth on acetate by modifying the acetate assimilation pathway. Xiao et al. (2013) overexpressed *acs* and *pta-ackA* in *E. coli* BL21(DE3), individually as well as in combination (Xiao et al., 2013). The highest cell growth and tolerance (at 100 mM acetate) was exhibited when *acs* was overexpressed alone. The strain with upregulated *pta-ackA* could grow well at 25 and 50 mM but only marginally at 100 mM. Noh et al. (2018) conducted a similar study and reported a 1.54-fold higher cell biomass following *acs* upregulation but a significant 3.85-fold lower biomass following the upregulation of *pta-ackA* (Noh et al., 2018). Similarly, Lin et al. (2006) reported that recombinant *E. coli* that overexpressed *acs* efficiently assimilated both externally supplemented acetate and self-secreted acetate and grew better (Lin et al., 2006). ACSs from different sources (*E. coli*, *Salmonella typhimurium* LT2, and *Acetobacter pasteurianus*) were assessed in *E. coli*. The ACS from *A. pasteurianus* was the best for cell growth among the three, although their expression levels have not been determined (Yang and Nie, 2016). On the other hand, in contrast to these positive results following *acs* upregulation, numerous negative results have also been reported. Huang et al. (2018) observed that the overexpression of *acs* seriously deteriorated cell growth and reduced the production of succinate, the target product (Huang et al., 2018). Instead, the upregulation of the *pta-ackA* pathway promoted acetate assimilation and succinate production. Similarly, Song et al. (2018) observed that the upregulation of the *pta-ackA* pathway promoted cell growth and acetate assimilation (Song et al., 2018). Li et al. (2016) also reported that the overexpression of *acs*, either from *E. coli* or *Bacillus subtilis*, in recombinant *E. coli* strains seriously deteriorated cell growth (Li et al., 2016). Novak and Pflugl et al. (2018) studied the overexpression of the mutant ACS enzyme, which is resistant to acetylation (Novak and Pflugl, 2018). The recombinant strain harboring the mutant *acs* showed no significant change in growth; the specific growth rate remained constant at 0.18 h^{-1} and the biomass yield decreased slightly to 0.26 (C_{mol}/C_{mol}). In a study on the production of isobutanol utilizing both glucose and acetate, Tashiro et al. (2015) observed that ACKA-PTA is more efficient than ACS (Tashiro et al., 2015). They suggested that, although PTA is a reversible enzyme and its K_m value for acetate is much higher (7 mM vs. 0.2 mM of ACS), ACKA-PTA is better when the acetate concentrations are high. These confusing and even contradictory results indicate that the improvement of acetate assimilation is not a simple task. It is likely that in addition to the conversion of acetate to acetyl-CoA by either *acs* or *pta-ack*, further metabolisms of acetyl-CoA and/or the maintenance of cellular homeostasis for acetyl-CoA and CoA-SH also affect the rate of acetate assimilation.

Adaptive laboratory evolution (ALE) has been employed to improve the tolerance to acetate and achieve better cell growth and acetate consumption, especially with *E. coli*. ALE has been efficient for developing strains that are tolerant to ethanol, n-butanol, 3-hydroxypropionic acid, high temperature, and/or pH (Ghiaci et al., 2013; Nguyen-Vo et al., 2019; Riehle et al., 2003; Trinh and Srienc, 2009). It has also been applied to increase the range of substrate utilization or their consumption rate (Lee and Palsson, 2010; Sanchez et al., 2010; Wisselink et al., 2009) and even the activation of latent pathways (Fong et al., 2006). Ibarra et al. (2002) evolved *E. coli* K12 on acetate by batch cultivation and, after ~700 generations, obtained a strain showing an increased growth rate by 20% with a concomitant increase in the oxygen and acetate uptake rates (Ibarra et al., 2002). Fong et al. (2003) evolved *E. coli* K12

on different substrates (such as lactate, pyruvate, and α-ketoglutarate) by batch cultivation for up to 625–1000 generations and observed an increase (by 13–138%) in the specific growth rate on these substrates with acetate (Fong et al., 2003). Chemostat culture was also employed for ALE. Rajaraman et al. (2016) obtained two evolved *E. coli* strains—MEC134 and MEC136—from *E. coli* ATCC8739 by progressively increasing dilution rates. The evolved strains showed higher specific cell growth rates (0.50–0.51 h^{-1}) (Rajaraman et al., 2016).

4. POTENTIAL OF ACETATE AS A FEEDSTOCK

Acetate is easily converted to acetyl-CoA, a key metabolite with which many metabolic pathways for cell growth and the production of useful metabolites are linked. Consequently, chemical compounds that can be synthesized from acetyl-CoA are good targets with acetate as the carbon source. For the transport and conversion of external acetate to internal acetyl-CoA, energy input is needed. Assuming that acetate is transported via passive diffusion of the free acid or by the proton (H^+)/monocarboxylate symporter (PMCT), one proton equivalent to 1/3 ATP (i.e., 3 protons are needed for the production of 1 ATP) is required. For the conversion of acetate to acetyl-CoA, one ATP molecule is used. However, when ACS, not ACKA-PTA, is the major route, the reaction proceeds through acetyl-AMP as the intermediate and AMP needs to be phosphorylated twice to be regenerated (see **2. Metabolism of acetate in microorganisms**). This means that, with ACS, two ATP molecules are required for the conversion of one molecule of acetate to acetyl-CoA. Therefore, the energy balance for the conversion of external acetate to internal acetyl-CoA (in) is (Zhou et al., 2020):

Acetate + CoA-SH + 1.33~2.33 ATP → Acetyl-CoA (in)

Acetate has several advantages as a carbon source. First, it can be used by diverse microorganisms. Second, acetate is fully miscible with water (thus different from gaseous substrates) and mass transfer is not a limiting factor during microbial fermentation. Third, owing to its oxidized nature, oxidized metabolites can be easily produced without the disposal of surplus reducing power. In addition, acetate can be used as an electron sink when co-metabolized with highly reduced substrates.

With acetate as a substrate and/or co-substrate, the production of a broad range of chemical compounds has been attempted (**Table 3**). Metabolic engineering strategies vary depending on host strains/organisms and/or target products, but they commonly include: the introduction and/or activation of the acetate assimilation pathway (*pta-ackA* and/or *acs*), modification of the glyoxylate cycle (by deleting *iclR* and/or increasing the pool of TCA/glyoxylate cycle intermediates), and/or (conditional) disruption of *pckA* (phosphoenolpyruvate carboxykinase) and *maeB* (malic enzyme) to suppress gluconeogenesis. In this chapter, we briefly describe the status of the use of acetate as a carbon feedstock and discuss metabolic and process engineering strategies (**Table 3**).

4.1 Succinic acid

Succinic acid is one of the top 12 value-added platform chemicals suggested by the US Department of Energy (DOE) (Bozell and Petersen, 2010). It has multiple applications including the synthesis of 1,4-butanediol, tetrahydrofuran, and γ-butyrolactone; it is also used as a monomer of biodegradable polymers (Cheng et al.,

2012). The synthesis of succinic acid requires low energy input owing to its oxidized nature; moreover, the relevant pathways such as the TCA cycle and glyoxylate shunt are well established in most microorganisms. Consequently, succinic acid should be considered a good target compound that can be produced from acetate. The material and energy balance for the production of succinate from acetyl-CoA is as follows:

$$2\ \text{Acetyl-CoA} \rightarrow \text{Succinate} + \text{NADH}$$

For simplicity, CoA-SH, H_2O, ADP, NAD^+, etc., were omitted in this equation. The energy requirement for the transport of the produced succinate was also ignored (For other target compounds described below, the same simplification is applied).

Succinic acid production from acetate has been studied well by Li et al. (Li et al., 2016). They used *E. coli* MG1655 as the host and engineered the strain by the disruption of the TCA cycle, activation of the glyoxylate shunt, and diversion of the carbon flux toward succinate (**Fig. 5A**). When succinate dehydrogenase was inactivated by disrupting *sdhAB*, 0.31 g/L succinate was produced from 0.73 g/L acetate. The additional deletion of *iclR*, the repressor of the glyoxylate cycle, was not very helpful, whereas the deletion of *maeB* (encoding malic enzyme, a key enzyme for gluconeogenesis) greatly improved succinate production (to 0.81 g/L from 1.2 g/L acetate). When citrate synthase (CS; encoded by *gltA*) was overexpressed in the triple mutant (*sdhAB*, *iclR*, *maeB*), succinate production was further improved to 1.94 g/L with a yield of 0.9 g/g acetate (**Table 3**), i.e., ~92% of the maximum theoretical yield. In a subsequent study (Huang et al., 2018), Huang et al. examined the effect of *pckA* (encoding PEPCK) deletion. The deletion of *pckA* was beneficial since it reduced the carbon flux toward gluconeogenesis and enhanced the availability of oxaloacetate, a precursor for succinate production. The quadruple deletion mutant (Δ*sdhAB* Δ*iclR* Δ*maeB* Δ*pckA*) produced 2.08 g/L succinate (0.87 g/g) and 2.78 g/L succinate when *gltA* was overexpressed. In this study, they found that ACKA-PTA is the main pathway for acetate uptake. Cells lacking *ackA-pta* grew poorly on acetate, and the double mutant (Δ*pta* Δ*acs*) could not grow on acetate at all. For a better assimilation of acetate, the native promoter of *ackA-pta* was replaced with a strong constitutive *trc* promoter (P_{trc}), which resulted in higher acetate consumption with a slightly enhanced succinate production (to 3.23 g/L). The overexpression of formate dehydrogenase (encoded by *fdh*) was also attempted for improving the NADH supply (Huang et al., 2018). Formate dehydrogenase regenerates NADH via formate oxidation by the following reaction:

$$\text{Formate} + NAD^+ \rightarrow \text{NADH} + CO_2 + H^+$$

With the exogenous supplementation of formate (at 10 mM), the recombinant strain (Δ*sdhAB* Δ*iclR* Δ*maeB* Δ*pckA ackA-pta*$^{++}$ *gltA*$^{++}$ *fdh*$^{++}$) formed 3.65 g/L succinate with a yield of 1 g/g acetate. Finally, a resting cell experiment was performed at high cell density (~50 OD_{600}). With appropriate feeding of acetic acid and formate, high succinate titer (194 mM or 22.91 g/L), yield (0.87 g/g acetate), and productivity (1.43 g/L/h) were achieved (**Table 3**). These results indicate that succinate is a promising target producible from acetate.

4.2 Itaconic acid

Itaconic acid (IA; 2-methylidenebutanedioic acid), an unsaturated dicarboxylic acid, is another top 12 value-added platform chemical selected by the US DOE (Werpy and Petersen, 2004). It can be used as a building block for fibers, resins, lattices, plastic, detergents, rubber, paint, surfactants, lubricants, and bioactive

compounds. It can also be used for the production of several other chemicals currently derived from petrochemical resources, such as acrylic acid, acetone cyanohydrin, maleic anhydride, and sodium tripolyphosphate (Bafana and Pandey, 2018; Steiger et al., 2013). Biologically, IA is produced via the decarboxylation of *cis/trans*-aconitate by the enzyme *cis*-aconitate decarboxylase (CAD) (**Fig. 5B**). *Cis/trans*-aconitate is a TCA cycle intermediate that appears when citrate is converted to isocitrate by the enzyme aconitase. To date, *Aspergillus terreus* is the most well-studied host for IA production, and the production of >80 g/L IA has been reported (Kuenz et al., 2012) with glucose as the carbon source. Similar to succinic acid, IA is suitable for production from acetate. The required energy input is low and the production pathway is simple when starting from acetyl-CoA. The material and energy balance for the production of IA is as follows:

$$3\ \text{Acetyl-CoA} \rightarrow \text{IA} + CO_2 + 2\ \text{NADH} + FADH_2$$

It has been assumed that succinate formed from the glyoxylate shunt is fully utilized to replenish oxaloacetate (OAA).

Only one study reported the use of acetate as the carbon source for IA production (Noh et al., 2018). In this work, the W strain of *E. coli* was selected as the host owing to its high growth rate (0.46 h^{-1}) and acetate consumption rate (0.22 g/g DCW/h). When CAD was overexpressed, recombinant *E. coli* W could produce IA at only a very low level (0.13 g/L). The upregulation of *acs* improved acetate assimilation, concomitant with higher biomass production; however, the increase in IA production was marginal (0.14 g/L). In comparison, the overexpression of *pta-ackA* caused a severe impairment in cell growth with no IA production. The deletion of *iclR* in a recombinant strain (Δ*iclR cad*$^{++}$ *acs*$^{++}$) improved the acetate uptake (1.33-fold), biomass formation (~2-fold), and IA production (0.59 g/L; 4.54-fold). The upregulation of *gltA* along with *aceA* (ICL) further improved IA production (to 0.84 g/L). Fed-batch cultivation of the recombinant strain (Δ*iclR cad*$^{++}$ *acs*$^{++}$ *glt*$^{++}$ *aceA*$^{++}$) yielded 6.24 g cdw/L and 3.57 g/L IA from 38.7 g/L acetate in 88 h (**Table 3**). The IA titer and yield are still very low compared to the values obtained using *A. terreus* grown on glucose as a carbon source.

4.3 3-Hydroxypropionic acid (3-HP)

3-Hydroxypropionic acid (3-HP) is also one of the top 12 value-added platform chemicals suggested by the US DOE (Bozell and Petersen, 2010). It is a structural isomer of lactic acid and can be converted to various chemicals such as acrylic acid, vinyl acetate, malonic acid, acrylamide, etc. 3-HP can also be used for the production of propiolactone, oligomers, and polyesters (Kumar et al., 2013). The biological production of 3-HP has been extensively studied with glucose or glycerol as the carbon source. With glucose as the carbon source, 3-HP can be produced from malonyl-CoA (an immediate precursor for fatty acid synthesis), which is synthesized from acetyl-CoA by the enzyme acetyl-CoA carboxylase. This means that the same pathway employed (using glucose as the carbon source) can be used for the production of 3-HP from acetate. To be converted to 3-HP, malonyl-CoA needs to be reduced by malonyl-CoA reductase (MCR) with NADPH as a cofactor via malonic semialdehyde (**Fig. 5C**). The pathway from malonyl-CoA to 3-HP is a part of the 3-hydroxypropionate and 3-hydroxypropionate/4-hydroxybutyrate cycles, two of the six pathways for autotrophic carbon dioxide fixation (Fuchs, 2011; Saini et al., 2011). Similar to succinic acid and IA, 3-HP is considered a good target owing to

its low energy input and simple production pathway. The material and energy balance for the production of 3-HP are as follows:

$$\text{Acetyl-CoA} + CO_2 + \text{ATP} + 2\ \text{NAD(P)H} \rightarrow \text{3-HP}$$

Two reports on 3-HP production from acetate have been published recently (Lee et al., 2018; Zhou et al., 2020). Lee et al. used *E. coli* as the host and introduced the MCR of *Chloroflexus aurantiacus* (Ca_*mcr*) to the host. In addition, *acs* was overexpressed to enhance acetate consumption and *iclR* was removed to activate the glyoxylate cycle (Lee et al., 2018). The recombinant *E. coli* (Δ*iclR mcr*$^{++}$ *acs*$^{++}$) could produce 3-HP at 0.25 g/L. Furthermore, upon adding cerulenin (50 μM) to the culture medium, 3-HP production improved to 3.0 g/L from 8.98 g/L of acetate (yield, 0.33 g/g acetate) (**Table 3**). Cerulenin irreversibly inactivates FabB and FabF, two important enzymes of the fatty acid biosynthetic pathway, which competes for malonyl-CoA. Zhou et al. (2020) used *Pseudomonas denitrificans* as the host and overexpressed heterologous Ca_*mcr* and endogenous acetyl-CoA carboxylase (encoded by *accABCD*) (Zhou et al., 2020). *P. denitrificans* is different from *E. coli* in that, for assimilation of acetate, succinly-CoA:acetate CoA-transferase (SCACT) takes CoA from succinyl-CoA and transfers it to acetate. Recombinant *P. denitrificans* overexpressing *mcr* and *accABCD* produced 1.74 g/L 3-HP when cerulenin was added and 1.28 g/L when *fabF* was deleted. Furthermore, non-growing cells devoid of *fabF* could continuously produce 3-HP up to 3.6 g/L, without losing production activity for 22 h. The impact of the glyoxylate shunt and TCA cycle on acetate consumption and 3-HP production was also studied by deleting *iclR* and/or *aceK*. The deletion of *iclR* slightly improved cell growth and acetate consumption (~5%) but significantly altered 3-HP production. In contrast, the *aceK* deletion mutant showed reduced cell growth (~8%) and acetate consumption (~6%) but an improved 3-HP yield on acetate (~8%). Thus, it was suggested that *iclR* deletion promotes acetate assimilation and gluconeogenesis by activating the glyoxylate shunt, whereas *aceK* deletion pushes more carbon toward the oxidative TCA cycle at the isocitrate node, thus increasing the availability of NAD(P)H and A(G)TP. Despite the fact that 3-HP is suitable for production from acetate, the titer and productivity are still far below commercially interesting levels.

More recently, Lama et al. (2021) developed GS-upregulated *E. coli* (Δ*poxB*Δ*adhE*Δ*ldhA*Δ*iclR mcr*$^{++}$) that produced 6.49 g/L 3-HP with yield of 0.39 g/g acetate and productivity of 0.15 g/L/h in two-stage cultivation mode. The TRY are the highest ever in 3-HP production from acetate.

4.4 Mevalonic acid (MA) and β-caryophyllene

MA (3,5–dihydroxy-3-methylpentanoic acid) and β-caryophyllene are described together in this section (**Fig. 5D-E**). MA is a C_6 compound; it is converted to isopentyl pyrophosphate (IPP), which has great industrial importance as a precursor in the synthesis of steroids and terpenes such as isoprene, pinene, carotenoid, artemisinin, and paclitaxel. β-Caryophyllene, synthesized from MA, is a common bicyclic sesquiterpene ($C_{15}H_{24}$) with potential as a next-generation aviation fuel. The biochemical pathway for MA synthesis starts with the condensation of two acetyl-CoA molecules to acetoacetyl-CoA, which further reacts with another acetyl-CoA molecule to produce hydroxylmethylglutaryl-CoA (HMG-CoA) and MA (**Fig. 5D**). The material and energy balance for the production of MA from acetyl-CoA are as follows:

$$3\ \text{Acetyl-CoA} + 2\ \text{NADH} \rightarrow \text{MA}$$

A study on MA production from acetate has been conducted by Xu et al. (2018). They generated recombinant *E. coli* by introducing the MA pathway comprising *mvaE* (acetyl-CoA acetyltransferase) and *mvaS* (HMG-CoA synthase), both derived from *Enterococcus faecalis*. In addition, acetyl-CoA synthase (*acs*) was also overexpressed in this recombinant strain. The resulting strain could produce 368 mg/L MA on a flask scale. In a two-stage fed-batch fermentation process, where the cells were initially grown on glucose (for 9 h to reach 8.6 g dcw/L) and then fed with ammonium acetate, the strain could produce 7.85 g/L MA with a yield of 0.27 g/g and productivity of 0.13 g/L/h, in a total time of 60 h (**Table 3**). The production of MA from acetate is straightforward, but the production levels are not yet high.

β-Caryophyllene is a constituent of essential oils commonly found in many plants, including clove, hop, rosemary, and basil (Harvey et al., 2014; Knudsen et al., 1993). It is known to have anti-carcinogenic and anti-inflammatory activities. Conventionally, β-caryophyllene is extracted from plants; however, the production yield is very low (Chang and Keasling, 2006; Maury et al., 2005). The material and energy balance for the production of β-caryophyllene from MA can be described in two steps—one from MA to IPP and the other from IPP to β-caryophyllene:

$$3\ MA + 9\ ATP \rightarrow 3\ IPP + 3\ CO_2$$

$$3\ IPP \rightarrow \beta\text{-caryophyllene} + 3\ PP_i$$

The synthesis of β-caryophyllene from acetate was conducted in recombinant *E. coli* (Yang and Nie, 2016). *E. coli* harbors the MEP (methylerythritol 4-phosphate; known as “non-mevalonate”) pathway for the synthesis of isoprenoid precursors—isopentenyl diphosphate (IDP) and dimethylallyl diphosphate (DMADP)—but lacks the enzyme β-caryophyllene synthase (QHS1) (**Fig. 5E**). The introduction of QHS1 (from *Artemisia annua*) along with the overexpression of *acs* (from *Acetobacter pasteurianus*) generated 56 µg/L, and the production was improved to 102 µg/L following the co-expression of geranyl diphosphate (GPP) synthase (from *Abies grandis*). They also introduced bridging genes for the conversion of acetyl-CoA to GPP, i.e., genes of the mevalonate pathway (from *Enterococcus faecalis*) and non-mevalonate pathway (from *Saccharomyces cerevisiae*), and acetoacetyl-CoA synthase (from *Streptomyces* sp.). The resulting recombinant exhibited improved β-caryophyllene production (up to 8 mg/L). The highest concentration of β-caryophyllene obtained by fed-batch cultivation of the recombinant *E. coli* strain was 1.05 g/L in 72 h (**Table 3**); however, this is still too low for commercial attention.

4.5 Free fatty acids and lipids

Fatty acids (FA) are promising precursors for the biosynthesis of biodiesel (fatty acid methyl ester, FAME), fatty alcohols, alkanes, alkenes, PHA, etc. (Yu et al., 2014). The commonly employed metabolic engineering strategy for FA synthesis includes the overexpression of acetyl Co-A carboxylase and thioesterase and truncation of the β-oxidation pathway (Lu et al., 2008; Steen et al., 2010). FA synthesis is an energy-intensive process and requires many NAD(P)H and ATP molecules. The material and energy balance for the synthesis of a typical saturated fatty acid, C16 palmitic acid, from acetyl-CoA are as follows:

$$8\ \text{Acetyl-CoA} + 7\ ATP + 14\ NAD(P)H \rightarrow \text{Palmitic acid}$$

Xiao et al. (2013) reported that an *E. coli* strain that overexpressed thioesterase (*tesA*) and *acs* could produce 370 mg/L FA from acetate (Xiao et al., 2013). The production was improved by 20% after blocking FA degradation through *fadE* knockout. By fed-batch bioreactor cultivation with a rich medium supplemented with

yeast extract, the recombinant strain (Δ*fadE acs*++ *tesA*++) grew without lag and produced 0.9 g/L FA in 26 h. However, in minimal medium, the cells grew after a long lag of 50 h and produced only 0.45 g/L FA in the same fed-batch cultivation system. The FA obtained from rich medium were mainly medium-chain-length FA (C_8-C_{14}; ~68%), which are of great interest for the production of jet fuel and pharmaceuticals.

The production of microbial lipids from acetic acid was also reported. The FA composition of microbial lipids is comparable to that of vegetable oils used for the production of biodiesel. Consequently, microbial lipids are considered a possible alternative to plant oil for biodiesel production (Christophe et al., 2012). In addition to acetic acid, other volatile fatty acids (VFA) such as propionic and butyric acids have been used and few are worthy of mention. VFA are generally toxic to microorganisms beyond 5 g/L; thus, Fontanile et al. (2012) developed a two-stage fed-batch fermentation system using the oleaginous yeast *Yarrowia lipolytica,* where the yeast is first grown on glucose/glycerol as the carbon source to a high density; in the second stage, microbial lipids are produced from VFA (Fontanille et al., 2012). When using glucose/glycerol for growth and acetate for lipid production, 12~16 g/L lipids with a content of 38~40% (g/g DCW) were obtained. In another study, *Cryptococcus curvatus* was used and the operation conditions of the second stage feeding and C/N ratio were optimized using acetate and ammonium sulfate. A C/N ratio of 300 in the feed led to high lipid production (25 g/L), which is 5.68-fold higher than that observed in the growth phase (**Table 3**) (Beligon et al., 2015).

Hu et al. (2016) also developed an interesting two-stage, integrated bioprocess for lipid production (Hu et al., 2016). In the first stage, the syngas derived from the gasification of coal/natural gas/biomass was anaerobically converted into acetic acid by *Moorella thermoacetica*, an acetogen with high autotrophic flux to acetyl-CoA through an efficient Wood-Ljungdahl pathway. In the second stage, the produced acetic acid was aerobically converted into microbial lipids by engineered *Y. lipolytica*. This process utilized hollow fiber membrane filter reactors for the recycling of cells and the continuous removal of acetic acid. For the first stage, a bubble column reactor was used, which was operated in three phases to grow *M. thermoacetica* and produce acetate. In Phase I (0~93 h), the strain was grown on a CO/CO_2 mixture with a focus on high biomass production, which resulted in 10.3 OD with 12 g/L acetic acid (productivity, 0.6 g/L/h). In Phase II (93–218 h), H_2 and CO_2 were supplied to enhance acetic acid production, which resulted in 25 g/L acetic acid (productivity, 0.9 g/L/h). The highest acetic acid concentration was set below 25 g/L to avoid toxic effects. The use of H_2 as an electron donor in Phase II limits ATP production in *M. thermoacetica* and channels more acetyl-CoA toward acetic acid formation rather than biomass. In Phase III (after 218 h), liquid medium was fed, and the culture medium was continuously transferred to the second stage for lipid production. During this phase, acetic acid production continued in the first stage where the acetate concentration was maintained at ~25 g/L. All three phases were under continuous mode for gaseous substrates, whereas for liquid medium, the first two phases were in batch mode and the final (third) phase was under continuous mode. In the second stage (stirred tank bioreactor), *Y. lipolytica* could produce 18 g/L lipids with a lipid content of 36% using the acetate transferred from the first stage. To increase the lipid titer and content in the second stage, the authors also tried cell recycling. With the longer residence time via cell recycling and the removal of spent medium containing low acetate levels, a high lipid titer of 46 g/L and lipid content of 59% could be achieved (**Table 3**). This study demonstrates an interesting integrated

process for acetic acid production from a greenhouse C_1 gas and its subsequent conversion to value-added microbial oil.

4.6 Poly (3-hydroxyalkanoates)

Poly-3-hydroxyalkanoates (PHAs) have garnered considerable attention as biodegradable polymers (Philip et al., 2007) To date, the best-known PHA is the homopolymer of 3-hydroxybutyrate (poly-3-hydroxybutyrate, PHB), but this polymer has inferior physicochemical properties such as brittleness and fragility (Bauer and Owen, 1988; Organ, 1993). Copolymers synthesized along with other monomers such as 4-hydroxybutyrate, hydroxyvalerate, hydroxyhexanoate, and hydroxyoctanoate can improve the inferior properties of PHB. Another important problem with PHAs is their higher production cost compared to that of oil-derived plastics. The major contributor to the overall PHB production cost is the carbon substrate cost; thus, the use of carbon substrates that are cheaper than glucose or sucrose can be exploited. The microbial biosynthesis of PHB starts with the condensation of two molecules of acetyl-CoA to acetoacetyl-CoA, which is subsequently reduced to hydroxybutyryl-CoA at the expense of NAD(P)H. Then, hydroxybutyryl-CoA is polymerized to PHB without further energy input. The material and energy balance for the synthesis of the monomeric hydroxybutyryl-CoA from acetyl-CoA are as follows:

$$2\ \text{Acetyl-CoA} + \text{NAD(P)H} \rightarrow \text{hydroxybutyryl-CoA}$$

Dai et al. (2007) enriched glycogen-accumulating organisms (GAOs) from wastewater and used them to produce copolymers containing two monomers—3-hydroxyvalerate (3HV) and 3-hydroxy-2-methylvalerate (3HMV)—by supplementing acetate as the single carbon source under alternating anaerobic and aerobic conditions (Dai et al., 2007). The PHA accumulated by the GAOs under anaerobic conditions contained copolymers with 30~35 C-mol% of 3HV and 3HMV in the 3HB chain, leading to desirable physical properties in comparison with PHB or the commercially available biodegradable copolymer poly(3HB-co-3HV) (PHBV). In contrast, under aerobic conditions, the production of high 3HB monomers from acetate caused a gradual decrease in the 3HV fraction. The polymers synthesized under anaerobic conditions possessed lower melting points and crystallinity, higher molecular weights, and narrower molecular-weight distributions, compared to the aerobically produced polymers. The highest yield of polymers obtained was 0.41 g/g in 120 h.

4.7 Isobutyl acetate and others

Isobutyl acetate (IBA; 2-methylpropyl ethanoate) can be used as a solvent, flavoring agent, and fuel (Rodriguez et al., 2014). The chemical synthesis of IBA proceeds via the esterification of isobutanol with acetic acid, whereas its biological synthesis takes place via the condensation of isobutanol and acetyl-CoA (**Fig. 5F**). Both isobutanol and acetyl-CoA can be synthesized from glucose as the sole carbon source, but the use of acetate as a co-substrate makes the synthesis and optimization of the pathway easier and economical. This approach was employed by Tashiroet al. (2015); isobutanol was generated from glucose, whereas acetyl-CoA was produced from acetate (Tashiro et al., 2015). As shown in **Fig. 5F**, isobutanol production from glucose is a redox-balanced process as two reducing equivalents generated during glycolysis are consumed. If acetyl-CoA is to be produced from glucose, pyruvate needs to be decarboxylated (mainly by the pyruvate

dehydrogenase complex, PDHc); this causes the loss of carbon and a redox imbalance (i.e., surplus of NADH). Furthermore, the control of carbon fluxes at the pyruvate node for the synthesis of isobutanol and acetyl-CoA becomes complex. The authors tested three acetate-consuming pathways (ACKA-PTA, ACS, and AldB-MhpF) in the IBA-producing *E. coli* JCL260 strain and observed that ACKA-PTA is the most efficient. It was suggested that, although PTA is a reversible enzyme and its K_M value for acetate is quite high (7 mM), the high acetate concentration employed in the experiment pushed the reaction toward the formation of acetyl-CoA.

To improve IBA production, *pta-ackA* was overexpressed and the recombinant could accumulate 13.9 g/L when grown on a mixture of glucose (50 g/L) and acetate (10 g/L) (**Table 3**). According to the stoichiometry of the biochemical reaction for IBA synthesis, the molar ratio of isobutanol/glucose and acetate should be 1:1. However, acetate is inhibitory beyond 10 g/L for *E. coli*. To overcome this toxicity issue, acetate was maintained at 10 g/L by feeding every 24 h; this resulted in the further improvement of IBA production to 19.7 g/L in 120 h. The authors also tested the effect of PDHc on IBA production by deleting the *aceEF* gene in the JCL260 strain. The reduction in acetate generation due to glucose was expected to improve IBA production. However, IBA production was lower and the *aceEF* strain grew slowly on a lower concentration of glucose. To increase cell growth and IBA production, the addition of acetate and/or the overexpression of *pta-ackA* was attempted. However, cell growth and IBA production did not improve, indicating the importance of PDHc for IBA production in this particular strain. Overall, this study is a good example of acetate utilization as a co-substrate for biological production.

In addition to the abovementioned compounds, tyrosine, phloroglucinol, and ethanol were also explored as target products to be produced from acetate when used as a (co)-substrate (**Table 3**). The production was confirmed, but the titer, yield, and productivity were not high. More extensive studies are warranted.

5. CHALLENGES AND FUTURE PERSPECTIVES

Many challenges arise in the use of acetate as a carbon feedstock. The most important challenge is the availability of cheap acetate in a sufficient amount. Now, C_1 gases such as CO and CO_2 are considered the best resources for the production of acetate. They are generated in massive amounts and their capture and subsequent immobilization into a non-volatile form of carbon are urgently required for reducing global warming. Among the several chemical or biological options, biological conversion to acetate is promising, considering the rate, yield, and scalability. However, it has not yet been commercialized owing to many technical issues in strain development, bioreactor operation, and downstream processing. More extensive studies to solve these technical problems should be conducted.

Another challenge is the nature of acetate as a carbon feedstock. Acetate is toxic to microbial growth and has a lower energy content than conventional carbon substrates such as glucose, sucrose, glycerol, etc. The level at which toxic effects appear is below 5 g/L, which is far lower than the desired titer of >50 g/L for many bulk products obtainable via fermentation. Consequently, acetate should not be added all at once but fed gradually during fermentation. The oxidized nature of acetate is another challenge when highly reduced and/or high carbon-numbered products are targeted. The reduction of the carbonyl group of acetate and/or the linkage of carbon-carbon bonds for higher carbon compounds is energy intensive;

thus, the product yield (g/g acetate) is not high. This limits the spectrum of target compounds that can be obtained with acetate as a carbon feedstock. The metabolic engineering of the host strain for energy production is also challenging since NAD(P)H and ATP should be generated from the catabolism of acetate. The TCA cycle and ETC are the two most important hubs for energy generation. This means that carbon distribution at the acetyl-CoA node should ensure proper energy generation and the supply of a carbon skeleton for the target compounds. However, the operation of the glyoxylate shunt, gluconeogenesis, and even the TCA cycle renders metabolic engineering efforts to control carbon flux distribution at the acetyl-CoA node highly challenging.

Concerning bioprocess development, the slow microbial growth and low acetate assimilation rate are important issues. With acetate as the carbon source, the highest specific growth rate of *E. coli* is ~0.5 h^{-1} (even in the presence of yeast extract), which is ~4-fold slower than the 2.0 h^{-1} observed with LB medium. To overcome this slow growth, a two-stage strategy has been adopted (more favorable carbon sources such as sugars and glycerol are employed for fast cell growth in the first stage; then, acetate is supplied for target product formation) and has proven useful in the production of succinic acid, 3-HP, and lipids. However, even with elevated cell growth rates during the growth stage, the low productivity due to slow assimilation of acetate during the production stage still remains unsolved. To accelerate acetate utilization, every step from its entry into the cell to its conversion to acetyl-CoA and target products should be enhanced. More details in regard to improve microbial acetate utilization and product formation are given in terms of three categories such as (i) conversion of acetate to acetyl-CoA, (ii) conversion of acetyl-CoA to target molecules, and (iii) alteration of energy metabolism (**Table 4**). Acceleration of acetate utilization requires a better understanding of acetate metabolism. However, as described before, acetate metabolism is highly regulated and interwoven with other metabolic pathways at different levels, including transcription, translation, post-translation, and enzyme activity. Consequently, any alteration to enhance the production of a target compound should be made in the context of whole acetate metabolisms. Owing to this complexity, even the seemingly simple question about the selection of ACS or ACKA-PTA has not yet been answered.

For the commercialization of the aforementioned products, extensive studies to improve TRY (titer, rate, and yield) are warranted. To this end, the selection of appropriate strains and their improvement are needed. Furthermore, reactor design for efficient cell growth and product formation should be optimized. However, the selection of the right target compound(s), with high demand in the market, is the most important aspect for commercialization. Considering the nature of acetate, a bulk product, rather than a fine or specialty chemical, should have better prospects. In addition, when acetate is used as the sole carbon substrate, less energy-consuming, more oxidized products are preferred. Well-established and simple pathways are also desired as they allow easier pathway engineering and the production is less energy intensive. In this regard, succinic acid, IA, and 3-HP should be considered ideal candidates since they are oxidized bulk products and their biological production pathways have been well established.

6. CLOSING REMARKS

The biochemical, microbial, and biotechnological aspects of acetate metabolism and the use of acetate as carbon feedstock have been described. Acetate should be considered a promising carbon feedstock for the production of various bio-chemicals that are oxidized in nature and/or easily produced from well-established synthesis pathways involving acetyl-CoA. Several chemicals, including succinic acid, IA, and 3-HP, belong to this category, though studies on their economical production are still at the early stages. Further studies to improve production titer, rate (productivity), and yield, comparable to those from favorable carbon sources (i.e., glucose, sucrose, glycerol, etc), are needed. For each candidate product and/or selected host strain, a better understanding of the complex metabolism of acetate, including acetate assimilation, the glyoxylate shunt, the TCA cycle, and/or gluconeogenesis, is also needed.

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AUTHOR CONTRIBUTIONS

Y.K., S.L., D.A., and V.K. wrote the original draft and revised the manuscript. V.K and S.P. conceptualized, wrote, and reviewed the manuscript.

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2151 **Table 1.** Enzyme characteristics of acetate kinase (ACKA) and acetyl-CoA synthetase (ACS)

Acetate kinase (ACKA)					
Organisms[a] (A, archaea; B, bacteria; Y, yeast)	K_M acetate [mM]	K_M acetyl-P [mM][b]	pH optimum[b]	Enzyme assay[c]	Reference
B1	7 59	0.16	7.5, 7.4	A A	Fox and Roseman, 1986; Nakajima et al., 1978
B2	1.2 7	0.16	7.5	A A	Chittori et al., 2012; Fox and Roseman, 1986
B3	40	0.00026	6.5-7.1	B	Vigenshow et al., 1986; Van Dyk and LaRossa, 1987
B4	40	0.44	7	A	Bock et al., 1999
B5	100	3.3	7	B	Bowman et al., 1976; Griffith et al., 1979
B6	7.9	N.D.	N.D.	A	Reinscheid et al., 1999
B7	48	1.0	7.5	C	Knorr et al., 2001
B8	120	2.3	7.3	A	Nakajima et al., 1978
B9	135	N.D.	N.D.	B	Schaupp and Ljungdahl, 1994
B10	285	N.D.	7.2-8	N.C.[d]	Lin et al., 1998
B11	160 73	< 1.0 0.58	8 N.D.	B B	Diez-Gonzalez et al., 1996[e]; Winzer et al., 1997[f]
B12	38.5	0.1	N.D.	B	Kahane and Muhlrad, 1979
A1	1.5 22	N.D. N.D.	N.D. 7.0- 7.4	A B	Ingram-Smith et al., 2005; Aceti et al., 1988;
Acetyl-CoA sythetase (ACS)					
Organisms[a]	K_M acetate [mM]	K_M ATP or CoA [mM][b]	pH optimum[b]	Enzyme assay[c]	Reference
B1	0.2	0.15 for ATP 0.2 for CoA	8.5	E	Brown et al., 1977; Enjalbert et al., 2017
B2	6.05 40	0.77 for ATP 0.05 for CoA	7.5	D	Reger et al., 2007; Chan et al., 2011
B13 ACS1 ACS2	 4.5 3.3	N.D.	N.D.	E	You et al., 2017

ACS3	2.9				
B14	1.2	0.35 for CoA; 5.6 for ATP	8	F	Li et al., 2011
A2 ACS1	3.5	3.3 for ATP; 0.19 for CoA	N.D.	F	Ingram-Smith et al., 2007
A3 ACS2	1.7	2.9 for ATP 0.3 for CoA	N.D.	F	Ingram-Smith et al., 2007
Y1 *ACS1* *ACS2*	 0.32 8.8	 1.4 for ATP 1.3 for ATP	 N.D. N.D.	 E E	van den Berg et al., 1996; Takahashi et al., 2006

Note:

[a] Details of microorganisms

	Name	Belong to	Characteristics
B1	*Escherichia coli*	Gram-negative bacteria	Facultative anaerobic; K_M values reported for both ACKA and ACS
B2	*Salmonella typhimurium*		
B3	*Rhodopseudomonas palustris*	Gram-negative bacteria	Facultative aerobic
B4	*Thermotoga maritima*	Gram-negative bacteria	Hyperthermophilic, anaerobic
B5	*Veillonella alcalescens*	Gram-negative bacteria	Strictly anaerobic; lactate-fermenting
B6	*Corynebacterium glutamicum*	Gram-positive bacteria	Facultative anaerobic; an industrial microbe for the production of amino acids
B7	*Lactobacillus sanfranciscensis*	Gram-positive bacteria	Strictly heterofermentative; lactic acid bacteria; High Haldane factor ($V_{max\ ATP\ formation}/V_{max\ ADP\ formation}$)
B8	*Geobacillus stearothermophilus (formerly Bacillus stearothermophilus)*	Gram-positive bacteria	Thermophilic, aerobic; Producing heat-resistant spores and thermostable proteins
B9	*Moorella thermoacetica (formerly Clostridium thermoaceticum)*	Gram-positive bacteria	Thermophilic, strictly anaerobic, acetogenic
B10	*Clostridium thermocellum*	Gram-positive bacteria	Thermophilic, strictly anaerobic, acetogenic; A candidate for consolidated bioprocessing (CBP)
B11	*Clostridum acetobutylicum*	Gram-positive bacteria	Strictly anaerobic, acetogenic
B12	*Acheloplasma laidlawii*	Gram-negative	Small genome size of 1.5 Mbp

		bacteria	
B13	*Saccharopolyspora erythraea (formerly Streptomyces erythraeus)*	Gram-positive bacteria	Erythromycin A-producing; ACSs tightly regulated by GlnR (nitrogen response regulator) at the transcriptional and post-translational levels
B14	*Mycobacterium tuberculosis*	Gram-negative or gram-positive bacteria	Pathogenic; PTA-ACKA present for acetate metabolisms, but no kinetic data available[g]
A1	*Methanosarcina thermophila*	Archaea	Thermophilic, acetoclastic, methane-producing
A2	*Methanothermobacter thermautotrophicus*		
A3	*Archaeoglobus fulgidus*		
Y1	*Saccharomyces cerevisiae*	Yeast	ACS1: acetyl-CoA biosynthesis, repressed by glucose ACS2: Histone acetylation

[b] N.D., not determined

[c] Enzyme assays (ACKA, Methods A~C; ACS; Methods D~F)

A. PK-LDH coupled assay: Acetyl-P_i synthesis was monitored by coupling reaction to NADH oxidation with the use of pyruvate kinase (PK) and lactate dehydrogenase (LDH). The reaction was monitored spectrophotometrically at 340 nm.

B. Hydroxamate-mediated colorimetric assay: Acetyl-P_i formed was determined by sequentially converting it into acetyl-hydroxamate. A ferric chloride-hydroxamic acid complex formed was measured at 540 nm.

C. PTA-MDH-CS coupled assay: Acetyl-P_i synthesis was monitored by coupling reaction to NADH oxidation with the use of phosphotransacetylase (PTA), malate dehydrogenase (MDH), and citrate synthetase (CS) as coupled enzymes. The reaction was measured at 340 nm.

D. MK-PK-LDH coupled assay (Similar to Method A): AMP formed was monitored by coupling reaction to NADH oxidation with the use of myokinase (MK), PK, and LDH as coupled enzymes. The reaction was monitored at 340 nm.

E. Hydroxamate-mediated colorimetric assay (Similar to Method B): Acetyl-CoA formed was monitored by sequentially converting it into acetyl-hydroxamate. A ferric chloride-hydroxamic acid complex formed was measured at 540 nm.

F. MD-CS coupled assay (Similar to Method C): Acetyl-CoA formed was monitored by NAD reduction with the use of MDH and CS as coupled enzymes. The reaction was started with addition of acetate and monitored at 340 nm.

[d] N.C., not clear

[e] *Clostridium acetobutylicum* P262

[f] *Clostridium acetobutylicum* DSM 1731

[g] Rücker et al., 2015

2174 **Table 2. Cell growth on acetate**

Strains		Specific growth rate[a] (h^{-1})	Acetate consumption rate (g/g DCW/h)[f]	References
Azotobacter vinelandii		0.35	-	(Tauchert et al., 1990)
Corynebacterium glutamicum		0.32[b]	0.97	(Wendisch et al., 2000)
Escherichia coli	B	0.34-0.41	0.91	(Rajaraman et al., 2016); Unpublished[e]
	BL21	0.30-0.44	0.94	(Rajaraman et al., 2016); (Noh et al., 2018); Unpublished[e]
	C (ATCC8739)	0.41; 0.5-0.51[c]	-	(Rajaraman et al., 2016); Unpublished[e]
	C (ATCC13706)	0.34-0.43	0.93	
	C (CGSC3121)	0.27	-	
	DH1	0.30	-	
	JM105	0.30	-	
	MACH1	0.34	-	
	MC4100	0.33	-	(Oh et al., 2002)
	MG1655	0.23-0.57	0.13-0.90	(Rajaraman et al., 2016); (Noh et al., 2018); (Ibarra et al., 2002); Unpublished[e]
	PB11	0.18; 0.27[c]	-	(Sabido et al., 2014)
	W	0.43-0.46	0.22-0.82	(Noh et al., 2018); Unpublished[e]
	W3110	0.2-0.26	0.91	(Rajaraman et al., 2016); (Noh et al., 2018); Unpublished[e]

Klebsiella pneumoniae J2B	0.43	0.82	Unpublished[e]
Methylobacterium extorquens	0.068[d]	-	(Schneider et al., 2012)
Pseudomonas putida KT2440	0.21	0.20	(Yang et al., 2019)
Pseudomonas aeruginosa PH1	0.18	0.125	
Thiobacillus sp.	0.22	-	(Gottschal & Kuenen, 1980)

Note: Cell growth was conducted in acetate-minimal medium containing yeast extract or a second carbon source. Acetate concentration also varied. Rajaraman et al. (2016), 1.7 g/L citric acid and 6.8 g/L acetate; Noh et al. (2018), 2 g/L yeast extract and 10 g/L acetate; Unpublished, 1 g/L yeast extract and 4 g/L acetate; and Gittschal and Kuenen (1980), Ibarra et al. (2002), Schneider et al. (2012), Tauchert et al. (1990), and Yang et al. (2019), 0.3–10 g/L acetate.

[a] Under optimal acetate concentrations among tested, unless stated otherwise

[b] At 60 mM acetate

[c] After adaptive laboratory evolution

[d] At 5 mM acetate

[e] From our own measurements

[f] Not determined

Table 3. Fermentative production of high value products using acetate as a substrate/co-substrate

Product	Strain	Titer[a] (g/L)	Yield (g/g)	Productivity (g/L/h)	Remarks (Reference)
Acetate as the main carbon source					
Succinic acid	*E. coli* MG1655 Δ*sdhAB* Δ*iclR* Δ*maeB_gltA*	1.94	0.90	0.014	(Li et al., 2016)
	E. coli MG1655 *ΔpckA* Δ*sdhAB* Δ*iclR* Δ*maeB_gltA*	7.29	0.59	0.101	Resting cell (Li et al., 2016)
	E. coli BW25113 *ΔpckA* Δ*sdhAB* Δ*iclR* Δ*maeB_ackA-pta_gltA_fdh*	3.65	1.0	0.051	(Huang et al., 2018)
	E. coli BW25113 *ΔpckA* Δ*sdhAB* Δ*iclR* Δ*maeB* Δ*icdA_ackA-pta_gltA_fdh*	22.91	0.87	1.43	Resting cell (Huang et al., 2018)
Itaconic acid	*E. coli* W Δ*iclR _cad_acs_aceA_gltA*	3.57	0.092	0.041	(Noh et al., 2018)
3-Hydroxypropionic acid	*E. coli* BL21 Δ*iclR _mcr_acs*	3.0	0.33	0.063	(Lee et al., 2018)
	Pseudomonas denitrificansΔ3hpdhΔ3hibdhIVΔ3hibdhIΔpta-ackAΔfab _mcr_acc	3.6	0.25	0.16	Resting cell (Zhou et al., 2020)
	E. coli BL21*ΔpoxBΔadhEΔadhE_mcr*	1.17	0.39	0.048	(Lama et al., 2021)
Mavelonic acid	*E. coli* W3110 *mvaE _mvaS_acs*	7.85	0.27	0.13	(Xu et al., 2018)
Tyrosine	*E. coli* Mach1-T1R_*acs_pck_aceA*	0.70	0.07	0.023	(Jo et al., 2019)
Phloroglucinol	*In vitro* system - ACS:ACC:PhiD (3:5:1)	0.74	0.64	0.049	(Zhang et al.,

					2017)
Fatty acids	*E. coli* Δ*fadE_tesA_acs*	1.0	0.063	0.016	(Xiao et al., 2013)
Lipids	*Cryptococcus curvatus* ATCC 20509	25	0.23	0.36	(Beligon et al., 2015)
	Y. lipolytica ACC-DGA strain (po1g)	18	0.09	0.19	Acetate from syngas (Hu et al., 2016)
	Y. lipolytica ACC-DGA strain (po1g)	46	0.16	0.27	(Hu et al., 2016)
β-Caryophyllene	*E. coli* BL21(DE3) *mvaE mvaS nphT7 QHS1 ERG12 ERG8 ERG19 IDI1 ACS*	1.05	0.021	0.015	(Yang & Nie, 2016)
Polyhydroxyalkonates	Mixed culture of glycogen-accumulating organisms		0.41 g/g DCW in 120 h		(Dai et al., 2007)
	Acetate as a co-substrate				
Ethanol (Glucose + Xylose)	*S. cerevisiae* D452-2 Δ*ALD6_XYL1_XYL2_XKS1_adhE*	45	0.414	0.51	(Ye et al., 2016)
Isobutyl acetate (Glucose)	*E. coli* JCL260 *ack-pta alsS ilvCD kivd adhA atf1*	19.7	0.33	0.164	(Tashiro et al., 2015)
Fatty acids (Glucose + Xylose)	*E. coli* Δ*fadE_tesA_acs*	0.43	0.037	0.0043	(Xiao et al., 2013)
Lipids (Glucose/Glycerol)	*Y. lipolytica* strain MUCL 28849	12-16	0.13-0.17	0.16-0.33	(Fontanille et al., 2012)
3-Hydroxypropionic	*E. coli* BL21*ΔpoxBΔadhEΔadhEΔiclR_mcr*	6.49	0.39	0.15	(Lama et al.,

	acid	2021)

Table 4. Classification of metabolic engineering efforts to enhance acetate utilization and product formation

Categories	Target metabolite	Target genes or pathways	References
Conversion of acetate to acetyl-CoA	3-HP	Overexpression of *acs*; deletion of *ackA-pta*	Lee et al., 2018; Zhou et al., 2020
	Succinic acid	Overexpression of *ackA-pta*	Huang et al., 2018
	Itaconic acid	Overexpression of *acs*	Noh et al., 2018
	Mevalonic acid	Overexpression of *acs*	Xu et al., 2018
	Tyrosine	Overexpression of *acs*	Jo et al., 2019
	β-Caryophyllene	Overexpression of *acs*	Yang and Nie, 2016
	Fatty acids	Overexpression of *acs*	Xiao et al., 2013
	Isobutyl acetate	Overexpression of *ackA-pta*	Tashiro et al., 2015
	Polyhydroxyalkonates	Overexpression of *ackA-pta*	Chen et al., 2018
Conversion of acetyl-CoA to target metabolite	3-HP	Overexpression of *mcr*; overexpression of *acc* and *mcr*	Lee et al., 2018; Lama et al., 2021; Zhou et al., 2020
	Succinic acid	Overexpression of *gltA*	Li et al., 2016; Huang et al., 2018
	Itaconic acid	Overexpression of *gltA* and *cad*	Noh et al., 2018
	Mevalonic acid	Overexpression of *mvaE* and *mvaS*	Xu et al., 2018
	Tyrosine	Deletion of *tyrR*; synthetic expression[a] of *aroG*, *aroABCDEL*, *tyrA*, and *tyrB*	Jo et al., 2019
	β-Caryophyllene	Overexpression of *mvaE, mvaS, nphT7, QHS1, ERG12, ERG8, ERG19,* and *IDI1*	Yang and Nie, 2016
	Fatty aicds	Overexpression of *tesA*; deletion of *fadE*	Xiao et al., 2013
	Lipids	Overexpression of *acc1* and *dga1*	Hu et al., 2016
	Isobutyl acetate	Overexpression of *alsS, ilvCD, kivD, adhA,* and *atf1*	Tashiro et al., 2015
	Polyhydroxyalkonates	Overexpression of *phaCAB*, *pct*, and *prpP*	Chen et al., 2018
Alteration of energy metabolisms	3-HP	Deletion of *iclR*	Lee et al., 2018
	Succinic acid	Deletion of *iclR* and *sdhAB*; overexpression of *fdh*	Li et al., 2016; Huang et al., 2018
	Itaconic acid	Deletion of *iclR*; overexpression of *aceA*	Noh et al., 2018
	Tyrosine	Overexpression of *aceA*	Jo et al., 2019

[a] A synthetic constitutive promoter and a synthetic 5′-untranslated region (5′-UTR) to each gene of interest were introduced to control at both transcription and translation level

FIGURE LEGENDS

Figure 1. Potential chemical and biological methods for acetate production

Figure 2. Major metabolic pathways for acetate assimilation and its conversion to value-added biochemical compounds. Acetate can be assimilated by ACS, ACKA/PTA (both far right and top), or SCACT (far left and bottom). The chemicals explored as possible targets to be produced from acetate are highlighted in the green boxes. Abbreviations: PEP, phosphoenolpyruvate; CoASH, free coenzyme A; Acetyl-P, acetyl phosphate; Acetyl-AMP, acetyl-adenosine monophosphate; PDHc, pyruvate dehydrogenase complex; PFL, pyruvate formate lyase; POXB, pyruvate oxidase; PTA, phosphate acetyltransferase; ACKA, acetate kinase; ACS, acetyl-CoA synthetase; CS, citrate synthase; ACN, aconitase; ICD, isocitrate dehydrogenase; KGDH, α-ketoglutarate dehydrogenase; SCS, succinyl-CoA synthetase; SCACT, succinyl-CoA:acetate CoA-transferase; SDH, succinate dehydrogenase; FUM, fumarase; MDH, malate dehydrogenase; ACEK, isocitrate dehydrogenase kinase/phosphatase; ICL, isocitrate lyase; MS, malate synthase; ME, malic enzymes; PEPCK, phosphoenolpyruvate carboxykinase; 3-HP, 3-hydroxypropionic acid; PHB, poly-3-hydroxybutyrate. Succinate from isocitrate via isocitrate lyase (ICL; encoded by *aceA*); itaconic acid from *cis*-aconitate as an intermediate of the TCA cycle via *cis*-aconitate decarboxylase (*cadA*); 3-HP from acetyl-CoA via malonyl-CoA reductases (*mcr*); fatty acids from acetyl-CoA via thioesterase (*tesA*); phloroglucinol from acetyl-CoA via phloroglucinol synthase (*phiD*); mevalonate from acetyl-CoA via hydroxymethylglutaryl-CoA reductase (*hmgr*); β-caryophyllene from mevalonate via sequential mevalonate pathway enzymes; PHB from acetyl-CoA via PHB pathway enzymes (*phaABC*). Solid line, single biological reaction; dashed line, multiple-step reactions.

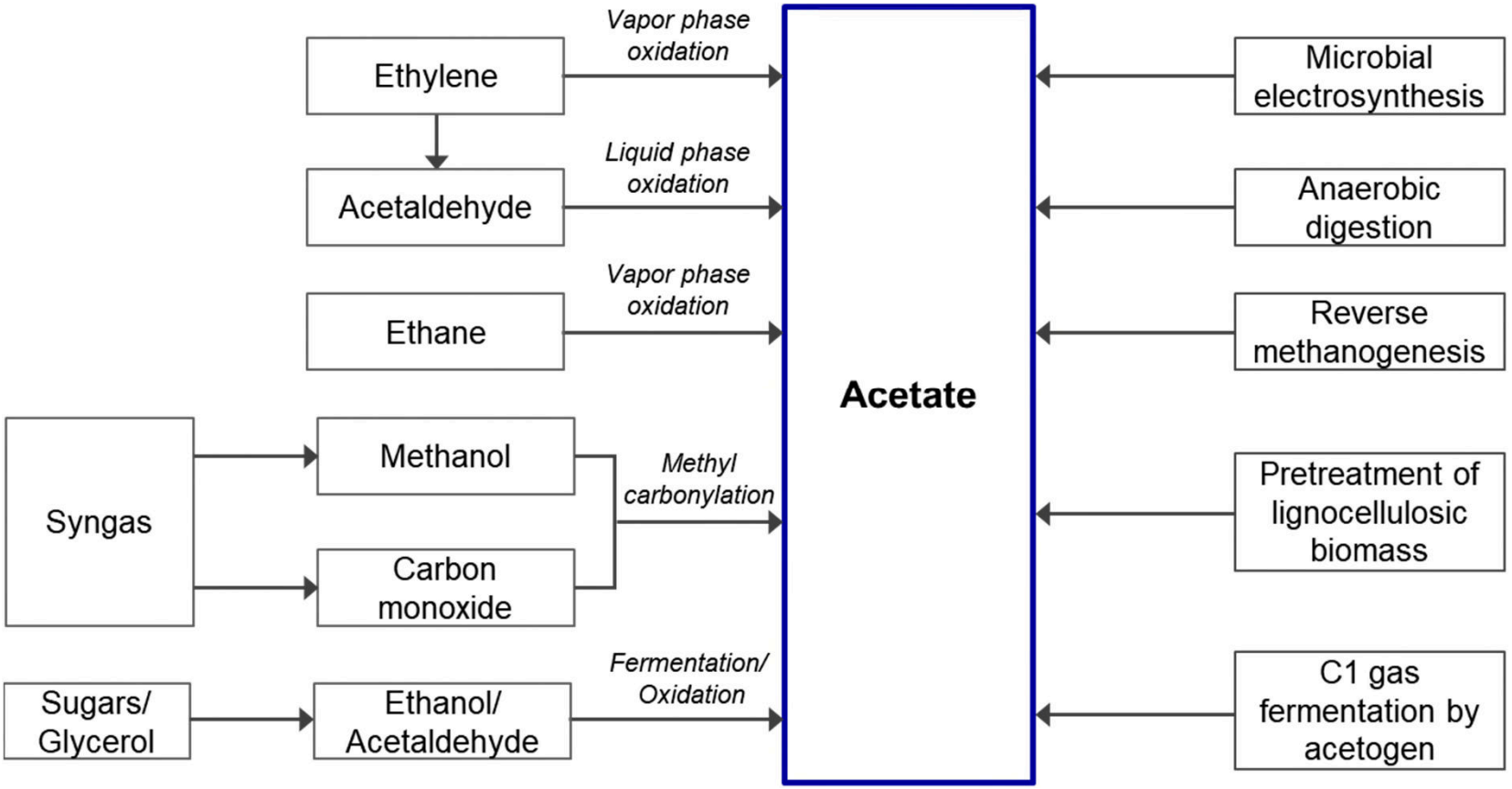
Figure 3. Regulation of acetate metabolisms at multiple levels. A, transcriptional regulation; B, posttranslational regulation; C, regulation by low-molecular-weight molecules. The regulation of the acetate scavenging pathway (ACS), the main acetate producing pathway (PTA-ACKA pathway), the *aar* operon (encoding succinyl-CoA:acetate CoA-transferase and mainly appearing in acetic acid bacteria and *Pseudomonas* sp.), the glyoxylate shunt (isocitrate lyase, ACEA and malate synthase, ACEB), and the isocitrate node (isocitrate dehydrogenase, ICD and its posttranslational regulator ICD kinase/phosphatase, ACEK) are depicted. In Panel A, the purple and orange ellipses indicate positive and negative controls, respectively. In Panel B, the reversible inactivation of enzymatic activity by acetylation (ACS-Ac, ACEA-Ac) or phosphorylation (ICD-P) are depicted. In Panel C, the red and blue lines indicate the enzymatic activity inhibition and activation, respectively, mediated by small molecules. The metabolically activated forms of acetate, acetyl-CoA and acetyl phosphate (acetyl-P), are donors of acetyl and phosphate groups for posttranslational protein acetylation and phosphorylation, respectively. Abbreviations: ACEA, isocitrate lyase; ACEB, malate synthase; ACEK, isocitrate dehydrogenase kinase/phosphatase; ACEK-K, ICD kinase; ACEK-P, ICD phosphatase; PTA, phosphotransacetylase; ACKA, acetate kinase; ACS, acetyl-CoA synthetase (AMP forming); ArcAB, aerobic respiration control protein (transcriptional regulator); Cra, catabolite repressor activator (FruR, transcriptional regulator); CreBC, carbon source responsive response regulator (transcriptional regulator); CRP, cAMP receptor protein (catabolite gene activator protein CAP, transcriptional regulator); IclR, glyoxylate bypass operon transcriptional repressor (transcriptional regulator); PatZ, peptidyl-lysine *N*-acetyltransferase; CobB, NAD+-dependent protein deacetylase (sirtuin); *actP*, acetate permease (gene); *yjcH*, a putative membrane protein in *acs-yjcH-actP* operon (gene); *icd*, isocitrate dehydrogenase (gene); ICD, isocitrate dehydrogenase (protein). (Modified from Bernal et al., 2016) (Bernal et al., 2016)

Figure 4. (A) Toxicity mechanism of acetate: At pH < pK_a, simple passive diffusion is employed for undissociated form only. At pH > pK_a, active transport is involved by symporters which are activated by proton (PMCT; H+/monocarboxylic acid symporter) or sodium (SSS; sodium:solute symporter). Once entering the cells, acetate dissociates into proton and anion, and disrupts cellular physiological functions by several effects classified as proton effects, anion effects, and intermediates effects. Anion-specific effect (i.e., *unknown*) is in italic.

(B) Tolerance mechanisms to acetate: Acetate enters the cell via passive diffusion (PT) and/or active transport (AT). The toxic effects of acetic acid are mitigated by various mechanisms, classified into five groups (gray boxes): (i) rapid acetate catabolism, (ii) transport of acetate via PMF-dependent antiporter and ABC transporter, (iii) amino acid decarboxylation to remove protons, (iv) change in cell morphology and membrane composition, and (v) expression of stress-induced regulons. Abbreviations: OM, outer membrane; PS, periplasmic space; IM, inner membrane; PMF, proton motive force; ABC, ATP-binding cassette; ΔpH, pH difference due to the H^+ concentration gradient; Δψ, membrane potential due to the electrical gradient; UFAs, unsaturated fatty acids.

Figure 5. Value-added products from acetate. The pathway enzymes and genes involved are depicted. (A) Succinic acid, enzymes, and genes involved: 1, acetyl-CoA synthetase (*acs*); 2, citrate synthase (*gltA*); 3, aconitase (*acnAB*); 4, isocitrate lyase (*aceA*). (B) Itaconic acid, enzymes, and genes involved: 1, acetyl-CoA synthetase (*acs*); 2, citrate synthase (*gltA*); 3, aconitase (*acnAB*); 4, *cis*-aconitate decarboxylase (*cad*). (C) 3-Hydroxypropionic acid, enzymes, and genes involved: 1, acetyl-CoA synthetase (*acs*); 2, acetyl-CoA carboxylase (*accABCD*); 3/4, malonyl-CoA reductase (*mcr*). (D) Mevalonate, enzymes, and genes involved: 1, acetyl-CoA synthetase (*acs*); 2, acetyl-CoA acetyltransferase (*mvaE*); 2, HMG-CoA synthase (*mvaS*); 4, HMG-CoA reductase (*hmgr*). (E) β-Caryophyllene, enzymes, and genes involved: 1, mevalonate kinase (*ERG12*), 2, phosphomevalonate kinase (*ERG8*); 3, mevalonate pyrophosphate decarboxylase (*ERG19*); 4, isopentenyl pyrophosphate isomerase (*IDI1*); 5, geranyl diphosphate synthase (*GPPS2*); 6, geranyl/Farnesyl diphosphate synthase (*ispA*); 7, β-caryophyllene synthase (*QHS1*). (F) Isobutyl acetate from the co-fermentation of glucose and acetate, enzymes, and genes involved: 1, 2-hydroxyl-3-ketol-acid reductoisomerase (*ilvC*); 2, dihydroxy-acid hydratase (*ilvD*); 3, 3-keto acid decarboxylase (*kivD*); 4, alcohol dehydrogenase (*adhA*); 5, acetate kinase (*ack*); 6, phosphotransacetylase (*pta*); 7, alcohol-O-acetyl transferase (*atf1*).

Figure 1.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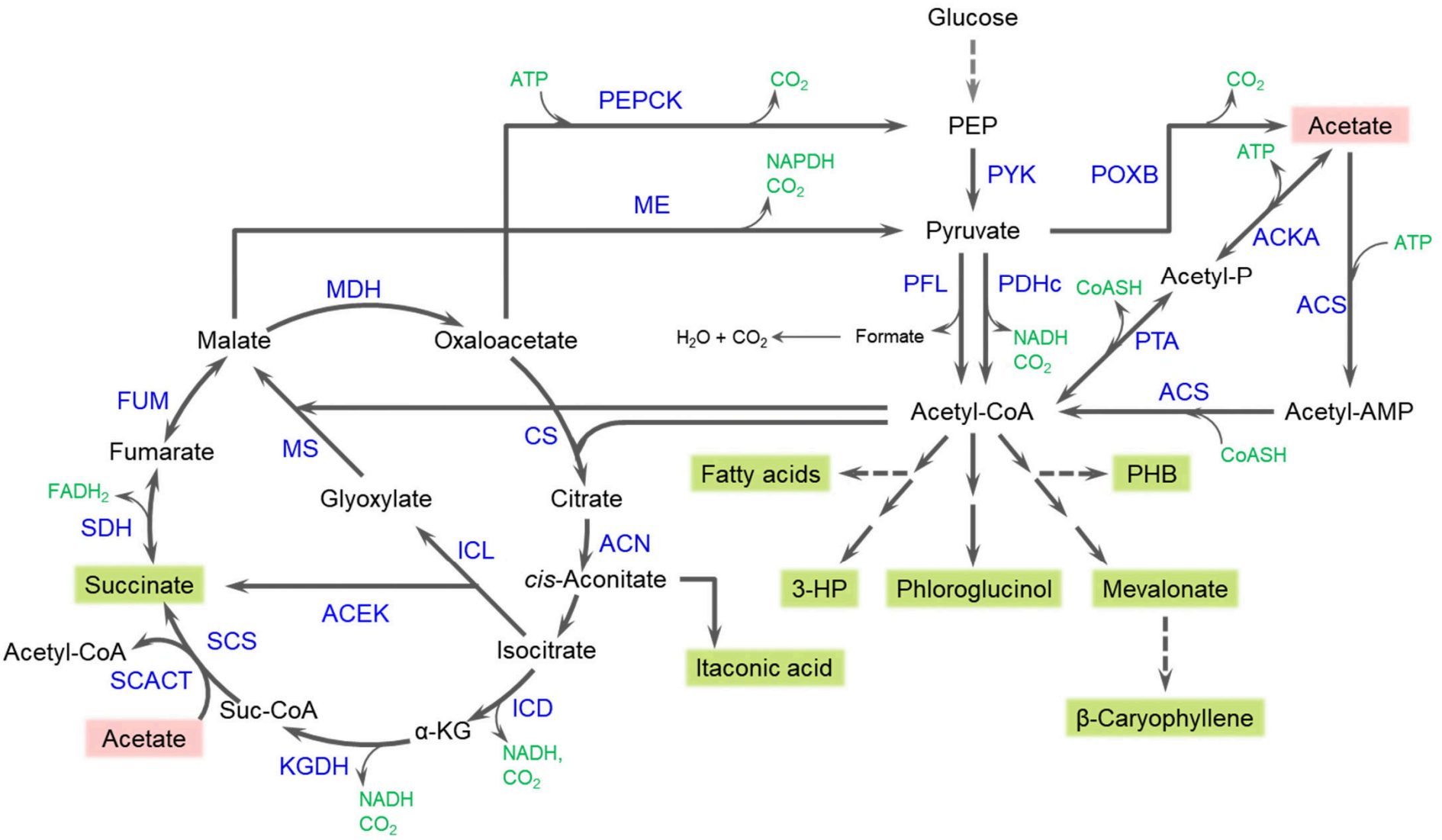
Acetaldehyde
Liquid phase oxidation
Ethane
Vapor phase oxidation
Syngas
Methanol
Carbon monoxide
Methyl carbonylation
Sugars/ Glycerol
Ethanol/ Acetaldehyde
Fermentation/ Oxidation
Acetate
Microbial electrosynthesis
Anaerobic digestion
Reverse methanogenesis
Pretreatment of lignocellulosic biomass
C1 gas fermentation by acetogen

Figure 2.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PEP
Pyruvate
Acetyl-CoA
Acetyl-P
Acetyl-AMP
Acetate
Malate
Oxaloacetate
Fumarate
Glyoxylate
Citrate
cis-Aconitate
Isocitrate
α-KG
Suc-CoA
Succinate
Acetyl-CoA
Formate
ATP
CO2
PEPCK
NAPDH
ME
PYK
POXB
ACKA
ACS
MDH
PFL
PDHc
CoASH
PTA
NADH
H2O + CO2
FUM
MS
CS
FADH2
SDH
ICL
ACN
ACEK
SCS
SCACT
ICD
KGDH
NADH, CO2
Fatty acids
PHB
3-HP
Phloroglucinol
Mevalonate
Itaconic acid
β-Caryophyllene

Figure 3.

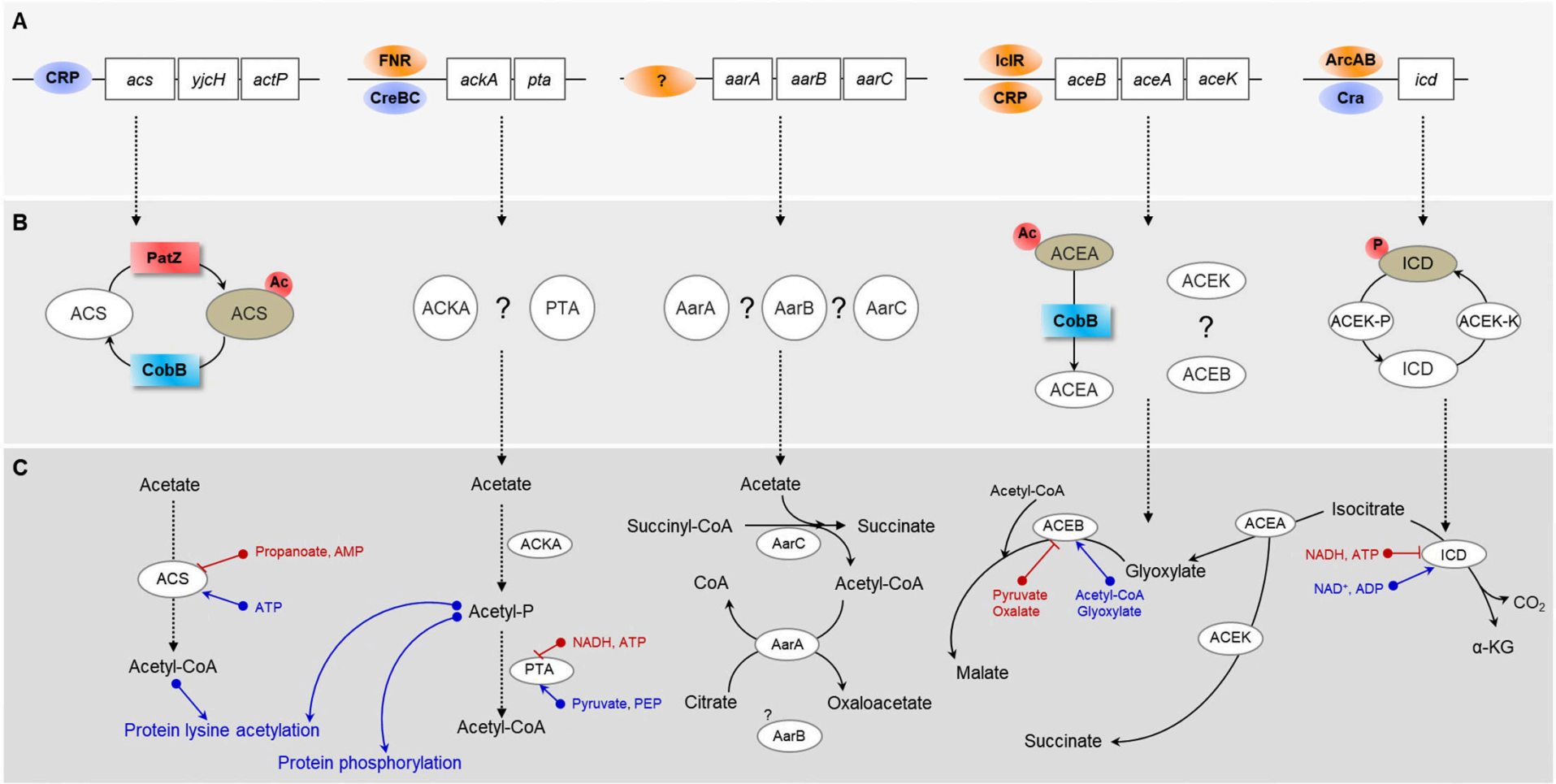
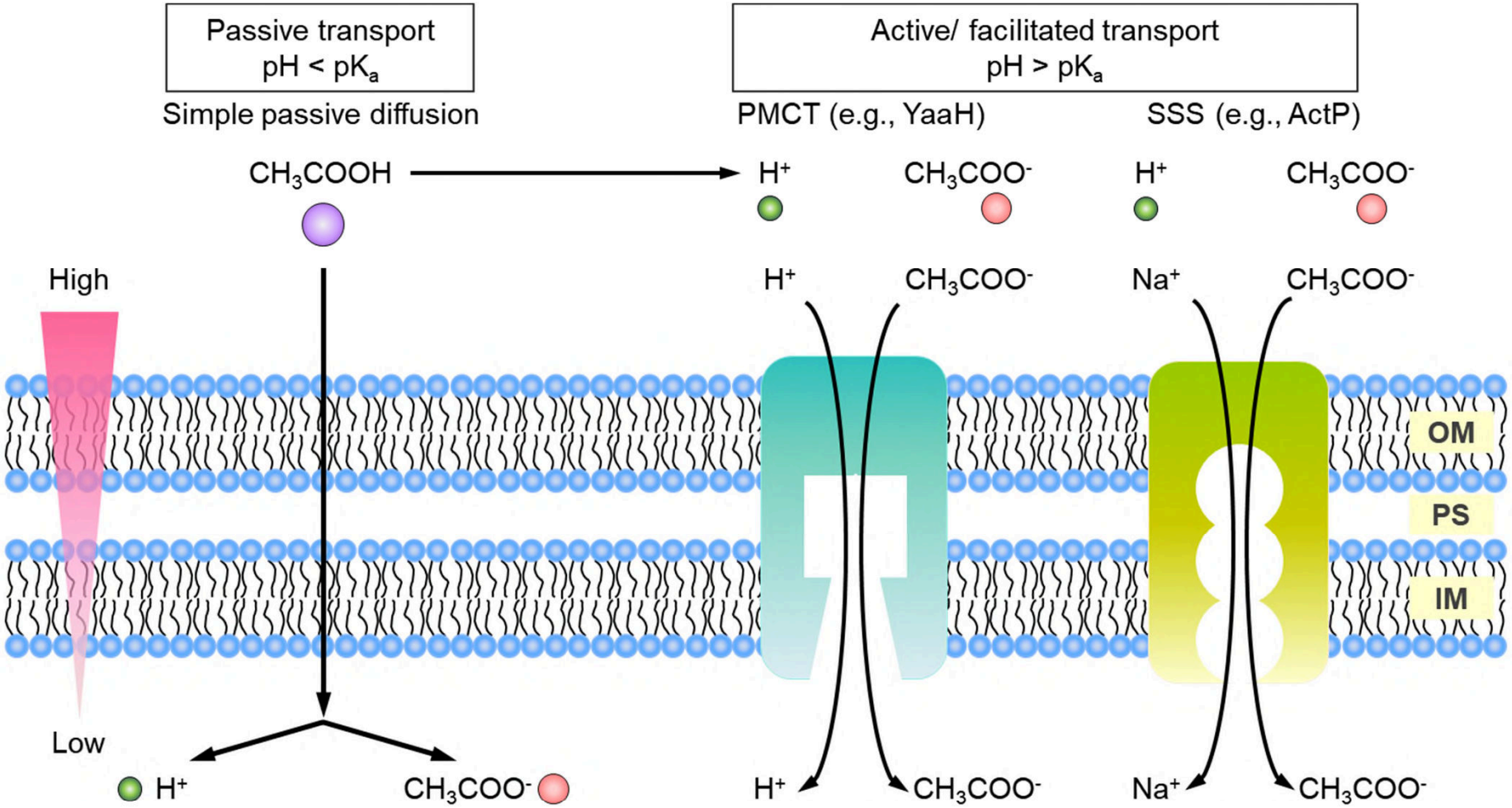

A
CRP
acs
yjcH
actP
FNR
CreBC
ackA
pta
?
aarA
aarB
aarC
IclR
CRP
aceB
aceA
aceK
ArcAB
Cra
icd
B
PatZ
Ac
ACS
ACS
CobB
ACKA
?
PTA
AarA
?
AarB
?
AarC
Ac
ACEA
CobB
ACEA
ACEK
?
ACEB
P
ICD
ACEK-P
ACEK-K
ICD
C
Acetate
ACS
Propanoate, AMP
ATP
Acetyl-CoA
Protein lysine acetylation
Acetate
ACKA
Acetyl-P
NADH, ATP
PTA
Pyruvate, PEP
Acetyl-CoA
Protein phosphorylation
Acetate
Succinyl-CoA
AarC
Succinate
CoA
Acetyl-CoA
AarA
Citrate
Oxaloacetate
?
AarB
Acetyl-CoA
ACEB
Pyruvate
Oxalate
Acetyl-CoA
Glyoxylate
Glyoxylate
Malate
ACEA
Isocitrate
NADH, ATP
ICD
NAD+, ADP
CO2
α-KG
ACEK
Succinate

Figure 4A.



Proton effects	Anion effects	Intermediate effects
• Intracellular acidification • Stress-induced regulon • Enzyme activity • Proton motive force	• Perturbation of anion pools • Turgor pressure • *Unknown*	• *Unknown*

Figure 4B.

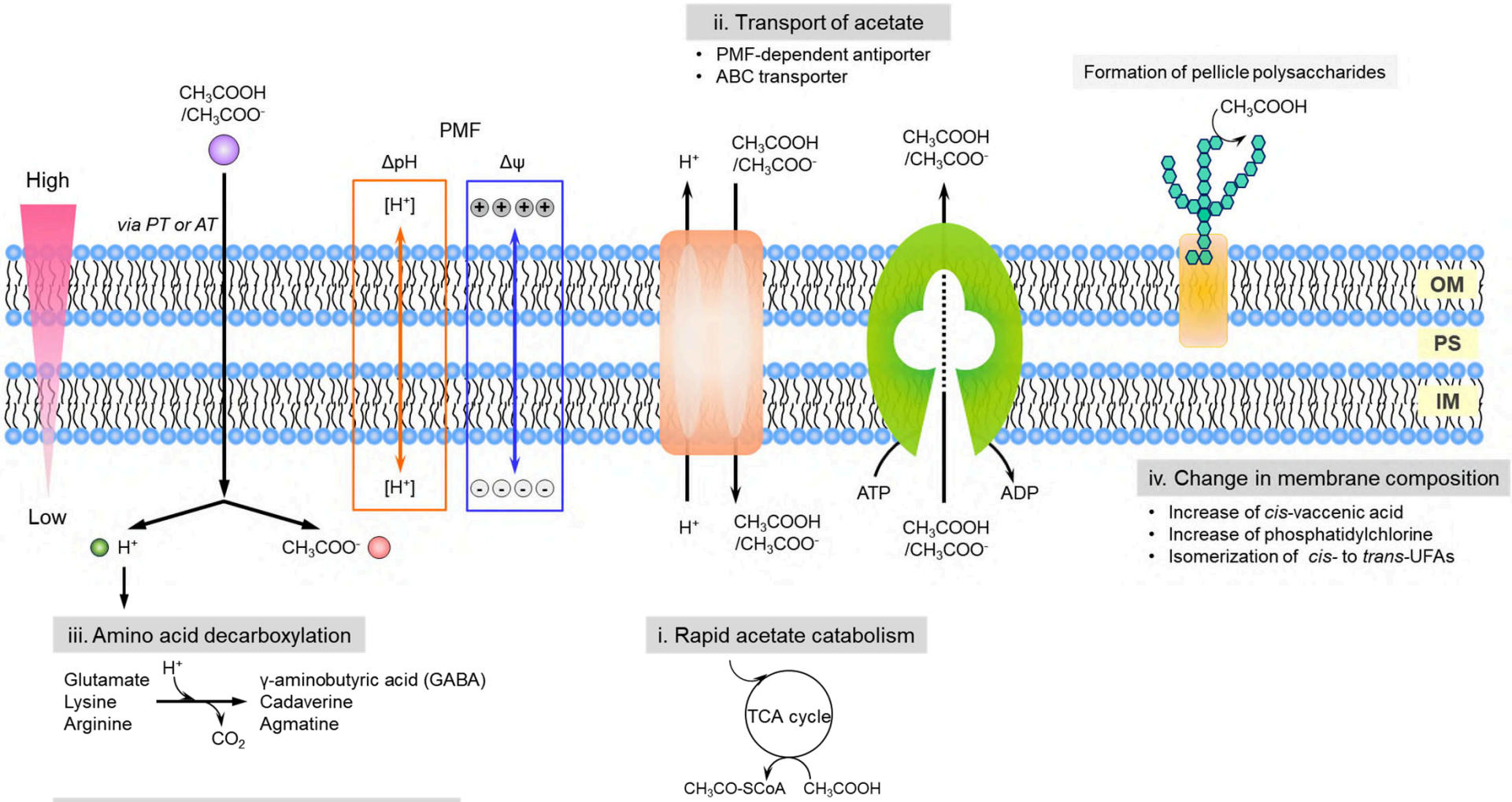
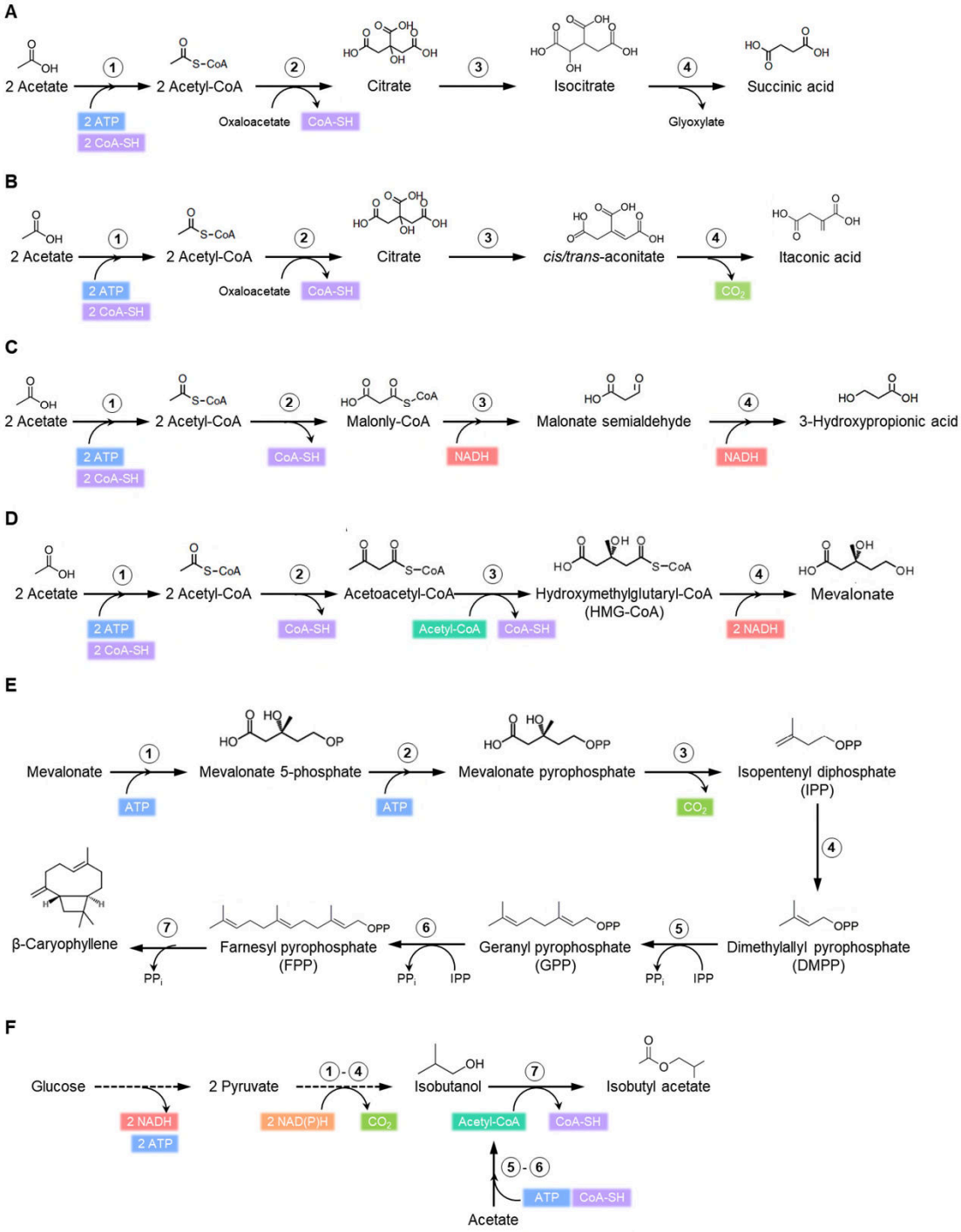

ii. Transport of acetate
• PMF-dependent antiporter
• ABC transporter
Formation of pellicle polysaccharides
CH3COOH
/CH3COO-
PMF
ΔpH
Δψ
High
via PT or AT
[H+]
[H+]
H+
CH3COOH
/CH3COO-
CH3COOH
/CH3COO-
CH3COOH
OM
PS
IM
Low
H+
CH3COO-
H+
CH3COOH
/CH3COO-
ATP
ADP
CH3COOH
/CH3COO-
iv. Change in membrane composition
• Increase of cis-vaccenic acid
• Increase of phosphatidylchlorine
• Isomerization of cis- to trans-UFAs
iii. Amino acid decarboxylation
Glutamate
Lysine
Arginine
H+
CO2
γ-aminobutyric acid (GABA)
Cadaverine
Agmatine
i. Rapid acetate catabolism
TCA cycle
CH3CO-SCoA
CH3COOH
v. Expression of stress-induced regulons
GroES, GroEL, DnaJ, DnaK, Hsp70, GrpE

Figure 5.


A
2 Acetate
2 Acetyl-CoA
Citrate
Isocitrate
Succinic acid
2 ATP
2 CoA-SH
Oxaloacetate
CoA-SH
Glyoxylate
B
2 Acetate
2 Acetyl-CoA
Citrate
cis/trans-aconitate
Itaconic acid
2 ATP
2 CoA-SH
Oxaloacetate
CoA-SH
CO2
C
2 Acetate
2 Acetyl-CoA
Malonly-CoA
Malonate semialdehyde
3-Hydroxypropionic acid
2 ATP
2 CoA-SH
CoA-SH
NADH
NADH
D
2 Acetate
2 Acetyl-CoA
Acetoacetyl-CoA
Hydroxymethylglutaryl-CoA
(HMG-CoA)
Mevalonate
2 ATP
2 CoA-SH
CoA-SH
Acetyl-CoA
CoA-SH
2 NADH
E
Mevalonate
Mevalonate 5-phosphate
Mevalonate pyrophosphate
Isopentenyl diphosphate
(IPP)
ATP
ATP
CO2
β-Caryophyllene
Farnesyl pyrophosphate
(FPP)
Geranyl pyrophosphate
(GPP)
Dimethylallyl pyrophosphate
(DMPP)
PPi
PPi
IPP
PPi
IPP
F
Glucose
2 Pyruvate
Isobutanol
Isobutyl acetate
2 NADH
2 ATP
2 NAD(P)H
CO2
Acetyl-CoA
CoA-SH
Acetate
ATP
CoA-SH