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MINI-REVIEW

# Acetate metabolism regulation in *Escherichia coli*: carbon overflow, pathogenicity, and beyond

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**Abstract** Acetate is ubiquitously found in natural environments. Its availability in the gut is high as a result of the fermentation of nutrients, and although it is rapidly absorbed by intestinal mucosa, it can also be used as carbon source by some members of gut microbiota. The metabolism of acetate in *Escherichia coli* has attracted the attention of the scientific community due to its role in central metabolism and its link to multiple physiological features. In this microorganism, acetate is involved directly or indirectly on the regulation of functional processes, such as motility, formation of biofilms, and responses to stress. Furthermore, it is a relevant nutrient in gut, where it serves additional roles, which regulate or, at least, modulate pathophysiological responses of *E. coli* and other bacteria. Acetate is one of the major by-products of anaerobic (fermenting) metabolism, and it is also produced under fully aerobic conditions. This acetate overflow is recognized as one of the major drawbacks limiting *E. coli*'s productivity in biotechnological processes. This review sums up current knowledge on acetate metabolism in *E. coli*, explaining the major milestones that have led to deciphering its complex regulation in the K-12 strain. Major differences in the metabolism of acetate in other strains will be underlined, with a focus on strains of biotechnological and biomedical interest.

**Keywords** Acetate overflow · Central metabolism · Catabolite repression · Pathogenic *E. coli* · Protein acetylation · Proteome reallocation

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## Introduction: acetate roles and functions

Acetate is ubiquitously found in natural environments, including gut, the natural niche of Enterobacteria (Cummings and Englyst 1987; Enjalbert et al. 2015; Macfarlane et al. 1992). The metabolism of acetate in *Escherichia coli* has attracted the attention of the scientific community due to its role in central metabolism and its link to multiple physiological features (Wolfe 2005). In gut, acetate is involved in the colonization and maintenance of microbial populations and acetate (and other short-chain fatty acids, SCFAs) regulate or, at least, modulate pathophysiological responses of *E. coli* and other bacteria (Herold et al. 2009; Lynnes et al. 2013; Ren et al. 2016; Sang et al. 2016). In biotechnological setups, acetate production even under fully aerobic conditions, is recognized as one of the major drawbacks limiting *E. coli*'s bioprocesses (Eiteman and Altman 2006; De Mey et al. 2007a).

In this minireview, current knowledge on acetate metabolism in *E. coli* is described, explaining its regulation and major differences between strains, with a focus on those of biotechnological and biomedical interests.

## *E. coli*: one species, several strains, different phenotypes

*E. coli* is the best known microorganism, and there is a wealth of information available on its physiology (Daegelen et al. 2009). Most works have focused on reference "laboratory strains" (such as the K-12 strain *E. coli* MG1655), which have been used systematically as workhorses in biology. Other strains, such as *E. coli* BL21, are known for being used for heterologous protein production. The specific features of the metabolism and physiology of other relevant strains are not that well known, which is especially true for pathogenic strains.

Classically, *E. coli* strains have been classified by their serotypes, e.g., being K-12 and BL21 serotype A or O157:H7 serotype E (Lukjancenko et al. 2010). The strain W (ATCC 9637), which is used nowadays for biotechnological purposes, is closely related with B1 serotype (Archer et al. 2011). A recent study, has classified 186 *E. coli* genomes based in the Homolog Gene Clusters. They observed that core genomes, including *Shigella*'s, were directly related with their serotypes. In fact, this last microorganism could be considered as an *E. coli* subspecies (Kaas et al. 2012). Interestingly, despite that acetate metabolism is closely connected to central metabolic pathways and its genes are highly conserved, it differs phenotypically in many of these *E. coli* strains.

## Acetate overflow: acetate metabolism is regulated at multiple levels

Acetate is one of the end products of the mixed acid fermentation metabolism of *E. coli*, but its production is not limited to $O_2$-deprived conditions (Caspi et al. 2014; Karp et al. 2014). When growing aerobically on glucose as the sole carbon source, glucose uptake and conversion into biomass and products are unbalanced, and significant amounts of acetate are excreted. This well-documented phenomenon is known as acetate overflow and has attracted the attention of microbiologists and biotechnologists for almost 40 years (Gleiser and Bauer 1981; Landwall and Holme 1977; De Mey et al. 2007b; Pan et al. 1987). Acetate overflow reduces biomass and product yields by consuming carbon equivalents, compromises the economy of industrial processes for the production of small-molecular-weight compounds (Eiteman and Altman 2006; De Mey et al. 2007b), and inhibits growth and the production of complex products such as proteins (Aristidou et al. 1995; Waegeman et al. 2013; Wong et al. 2008) or plasmid DNA (Borja et al. 2012; Cunningham et al. 2009).

The causes of acetate overflow are complex. It was initially believed that the phenomenon was caused by a limited respiratory capacity of cells, which would lead to the accumulation of reduced cofactors (NAD(P)H), tricarboxylic acid (TCA) cycle intermediates, and acetyl-CoA (Han et al. 1992; Varma and Palsson 1994). Although the problem is not completely understood, it is currently accepted that it is the result of several combined effects. Acetate metabolism regulation in *E. coli* is the result of the interplay between several interconnected and interregulated layers operating at different cellular levels: gene transcription, posttranscriptional regulation, posttranslational modification of proteins, modulation of protein activity by low-molecular-weight molecules, etc. (Basan et al. 2015; Castaño-Cerezo et al. 2014; Enjalbert et al. 2015; Peebo et al. 2015; Schilling et al. 2015; Valgepea et al. 2010). All these regulation mechanisms concur in the cell, but their contribution to the regulation of acetate metabolism in *E. coli* will be dissected separately for the sake of clarity.

## How is acetate metabolized in *E. coli*?

Acetate pathways are redundant, which underlines the importance of this metabolite for environmental growth. The relative importance of these pathways differs between aerobic and anaerobic conditions. Acetate production and consumption pathways in *E. coli* have been studied in depth in *E. coli* K-12. In this and the following sections, we will focus on the knowledge built around this model strain.

### Mechanisms of acetate transport through cell membranes

Thanks to its small size, acetate freely permeates the cell membrane in both the anionic and neutral charge forms (Axe and Bailey 1995). The permeation of acetic acid through the membrane dissipates the proton gradient and lowers the pH of the cytoplasm. In addition, it uncouples the proton-motive force, inhibiting ATP synthesis and growth of *E. coli* (Wolfe 2005).

Two acetate membrane transporters have been identified. The *actP* gene encodes a permease which is expressed from an operon which also encodes for acetyl-coenzyme A synthetase (*acs*), involved in acetate scavenging in the stationary phase (Fig. 1) (Gimenez et al. 2003). Giménez and colleagues suggested that ActP was not the only active acetate transporter in *E. coli*. Recently, the acetate/succinate symporter SatP (encoded by the *satP* gene) has been identified (Sá-Pessoa et al. 2013). The SatP symporter activity is linked to the exponential growth stage whereas the ActP permease is more active at the stationary phase (Gimenez et al. 2003; Sá-Pessoa et al. 2013). Both Δ*satP* and Δ*actP* strains show a partial decrease in the uptake of acetate while the Δ*satP*Δ*actP* double mutant shows a strong decrease in acetate uptake rate. This suggests that facilitated transmembrane transport is the major uptake mechanism in *E. coli*.

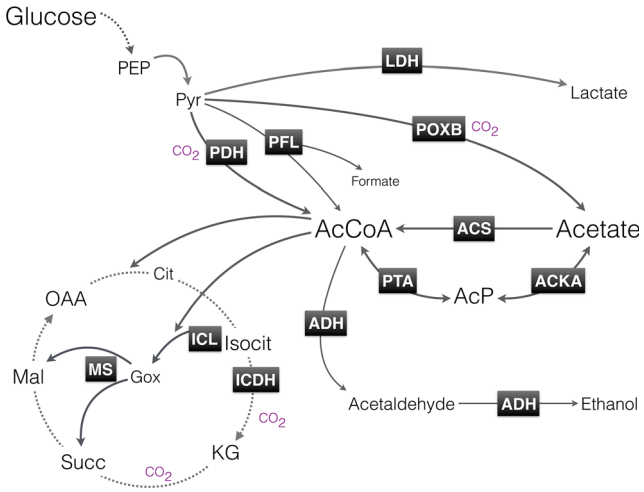


**Fig. 1** Major metabolic pathways of glucose metabolism in *E. coli*. The main pathways involved in the production and consumption of acetyl-CoA are depicted (see the text for details). *AcCoA* acetyl-CoA, *ACS* acetyl-CoA synthetase (AMP-forming), *ACKA* acetate kinase, *AcP* acetyl-phosphate, *ADH* aldehyde/alcohol dehydrogenase, *Cit* citrate, *OAA* oxaloacetate, *Gox* glyoxylate, *ICDH* isocitrate dehydrogenase, *ICL* isocitrate lyase, *Isocit* isocitrate, *KG* α-ketoglutarate, *LDH* lactate dehydrogenase, *Mal* malate, *MS* malate synthase, *PDH* pyruvate dehydrogenase complex, *PEP* phosphoenolpyruvate, *PFL* pyruvate formate lyase, *POXB* pyruvate oxidase, *PTA* phosphotransacetylase, *Pyr* pyruvate, *Succ* succinate

## Acetate production pathways: pyruvate dehydrogenase, pyruvate formate-lyase, pyruvate oxidase, and phosphotransacetylase/acetate kinase

Acetate is produced by oxidation of pyruvate via acetyl-CoA. The activated form of acetate, acetyl-CoA, is produced by oxidative decarboxylation of pyruvate, catalyzed by pyruvate dehydrogenase (Pdh) complex, and by pyruvate formate lyase (Pfl) under aerobic and anaerobic conditions, respectively (Fig. 1) (de Graef et al. 1999; Knappe and Sawers 1990).

During exponential growth, acetyl-CoA is transformed into acetate via acetyl-phosphate by the two step pathway catalyzed by phosphotransacetylase (Pta) and acetate kinase (AckA), encoded by the *ackA-pta* operon. This is a reversible and low-affinity pathway, which can also uptake acetate when present at high concentrations in the environment (Fig. 1). The levels of these enzymes are usually high, which makes it a high-capacity route (Castaño-Cerezo et al. 2009; Kakuda et al. 1994).

Transcription of *ackA-pta* is controlled through the transcriptional regulator of the transition from aerobic to anaerobic conditions (Fnr) and, at least partly, by the aerobic respiration control protein (ArcA) (Li et al. 2014; Shalel-Levanon et al. 2005). The *ackA-pta* transcriptional unit is part of the regulon of the two components system CreBC (Fig. 2). CreC is the histidine kinase membrane sensor of the system, which autophosphorylates in response to unknown environmental signals. The response element CreB is phosphorylated, and it binds to specific sequences (called *cre tag* binding sites) in the promoter region of several genes and operons, repressing or activating their transcription (Cariss et al. 2008). They are encoded by the *creABCD* operon, which transcription is activated during growth in minimal medium, when glycolytic carbon sources are being fermented or during aerobic growth when low-molecular-weight fermentation products are used as gluconeogenic carbon sources. In fact, the CreBC system responds to growth in minimal medium under the mentioned conditions (Avison et al. 2001; Cariss et al. 2008; Caspi et al. 2014; Godoy et al. 2015; Kakuda et al. 1994; Karp et al. 2014; Sprenger 1995). Moreover, the regulation mediated by CreBC is affected by oxygen availability: deletion of *creC or creB* affects acetate production and acetate kinase activity in microaerobiosis and anaerobiosis (Godoy et al. 2015).

Oxidative decarboxylation of pyruvate by pyruvate oxidase (PoxB) directly produces acetate (Fig. 1). PoxB is a peripheral membrane protein which couples substrate oxidation to the electron transport chain via ubiquinone. This is the major pathway for acetate production in the stationary phase and under phosphate starvation. In fact, the expression of *poxB* depends on the RpoS sigma factor, which is necessary for the transcription of many stationary phase-induced genes. It is believed that it may decrease oxidative stress and contributes to the metabolic efficiency of *E. coli*, a role which might be especially relevant under microaerobic conditions, where both pyruvate dehydrogenase and pyruvate formate lyase function is suboptimal (Abdel-Hamid et al. 2001; Chang et al. 1994).

## Acetate consumption: the high-affinity acetyl-CoA synthetase pathway

Acetate produced during exponential growth on glucose is uptaken upon glucose exhaustion. Acetyl-CoA synthetase (Acs) is a high-affinity enzyme used to scavenge small acetate concentrations (<10 mM) upon depletion of the main carbon source (Starai and Escalante-Semerena 2004). The function of this pathway is mainly catabolic. Accordingly, its expression is linked to that of the Pta-AckA and the glyoxylate shunt pathways (Kumari et al. 2000a), being directly regulated by catabolite repression and the nucleoid proteins Fis and IHF and indirectly by several other transcription factors (Karp et al. 2014; Sclavi et al. 2007; Valgepea et al. 2010). Expression of *acs* is negatively regulated by Fis in the exponential phase of growth and positively by cAMP-CRP in the stationary phase (Kumari et al. 2000a; Wolfe 2005). The carbon storage regulator system (CSR), which regulates biofilm formation, peptide uptake, motility, virulence, and carbohydrate storage, also regulates central carbon metabolism pathways. In fact, it controls phosphofructokinase (Pfk) and the upper part of glycolysis. Analysis of a *csrA*-attenuated mutant

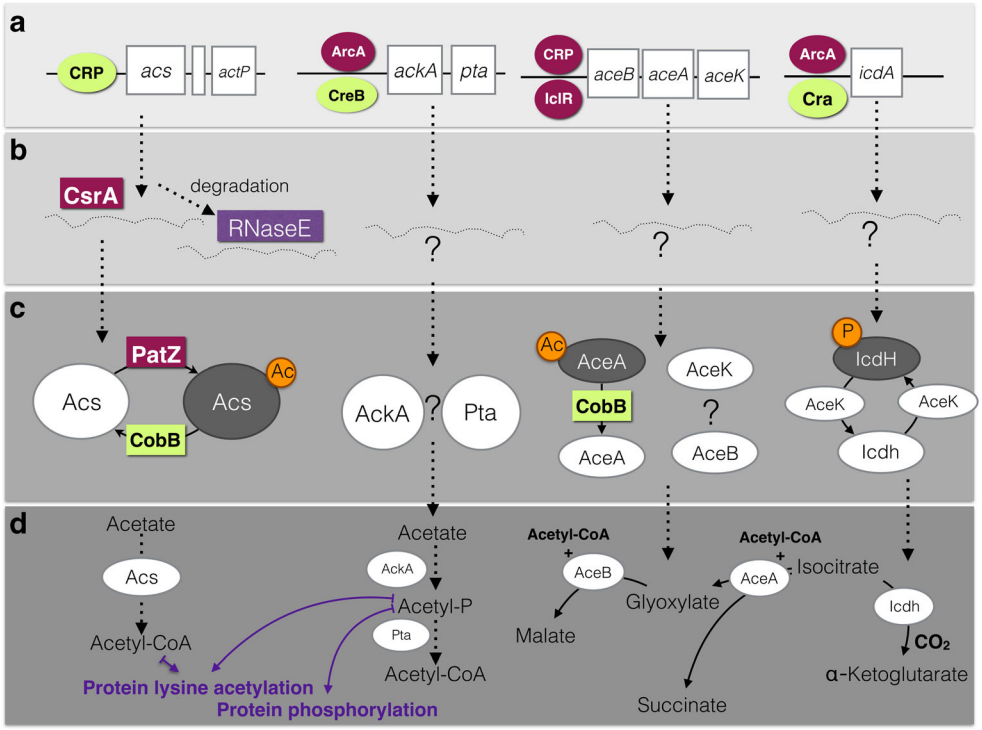


**Fig. 2** Multiple levels of regulation of acetate metabolism: **a** transcriptional, **b** posttranscriptional, **c** posttranslational, and **d** regulation by low-molecular-weight molecules. The regulation of the acetate scavenging pathway (acetyl-CoA synthetase, Acs), the main acetate producing pathway (Pta-AckA pathway), the glyoxylate shunt (isocitrate lyase AceA and malate synthase AceB), and the isocitrate node (isocitrate dehydrogenase Icdh and its posttranslational regulator Icdh phosphatase/kinase AceK) are depicted. The metabolically activated forms of acetate, acetyl-CoA and acetyl-P, are the donors of acetyl and phosphate groups for posttranslational protein acetylation and phosphorylation, respectively. *AceA* isocitrate lyase, *AceB* malate synthase, *AceK* isocitrate dehydrogenase phosphatase/kinase, *AckA* acetate kinase, *Acs* acetyl-CoA synthetase (AMP-forming), *ArcA* aerobic respiration control protein (transcriptional regulator), *Cra* catabolite repressor activator (FruR, transcriptional regulator), *CreB* carbon source responsive response regulator (transcriptional regulator), *CRP* cAMP receptor protein (catabolite gene activator protein CAP, transcriptional regulator), *CobB* $NAD^+$-dependent protein deacetylase (sirtuin), *icdA* isocitrate dehydrogenase (gene), *IcdH* isocitrate dehydrogenase (protein), *PatZ* protein acetyl-transferase, *Pta* phosphotransacetylase, *RNase E* ribonuclease E

has revealed an increase in acetyl-CoA synthetase levels, contributing to lowering acetate overflow (Esquerré et al. 2016; Morin et al. 2016).

Acetyl-coenzyme A synthetase is regulated by acetylation (Castaño-Cerezo et al. 2014; Starai et al. 2002) which will be further explained in the following sections. In continuous cultures, the coexpression of *acs* and *ackA-pta* leads to establishing a futile cycle of simultaneous acetate production and scavenging which limits acetate overflow at low growth rates (Fig. 1) (Renilla et al. 2012; Valgepea et al. 2010).

## Metabolic fates of acetyl-CoA: the TCA cycle and the glyoxylate shunt

Acetyl-CoA is substrate of several catabolic and anabolic pathways. The major fate of acetyl-CoA is the TCA cycle, which leads to its complete oxidation into $CO_2$, with concomitant generation of cellular energy as reduced cofactors. Consequently, acetate metabolization through the TCA cycle cannot allow biomass synthesis.

The glyoxylate shunt is a shortcut of the decarboxylating steps of the TCA cycle, allowing the use of acetyl-CoA for net synthesis of biomass constituents from two carbon substrates (Chung et al. 1988; Maloy and Nunn 1982). Isocitrate lyase and malate synthase enzymes are specific of the glyoxylate shunt, and they jointly convert isocitrate and acetyl-CoA into malate and succinate. These intermediaries of the TCA cycle are further metabolized by succinate dehydrogenase and malate dehydrogenase. The glyoxylate shunt is only present in bacteria and plants (Fig. 1). In *E. coli*, these enzymes are encoded by the *aceBAK* operon (Brice and Kornberg 1968).

Since the glyoxylate shunt avoids the decarboxylating steps of the TCA cycle, acetyl-CoA contributes to net biomass synthesis and this pathway is needed for growth on acetate or acetogenic substrates such as fatty acids. The glyoxylate shunt is normally inactive in the presence of glucose excess and acetyl-CoA is mainly oxidized in the TCA cycle (Walsh and Koshland 1984).

In addition to its classically known anabolic role, $^{13}C$-based flux analysis has recently revealed that the glyoxylate shunt is active as part of a cyclic catabolic pathway: the PEP-

glyoxylate cycle. This novel pathway involves pyruvate dehydrogenase, phosphoenolpyruvate carboxykinase, and the glyoxylate shunt, which jointly allow the complete oxidation of acetyl-CoA to $CO_2$ while avoiding NADPH synthesis in the oxidative steps of the TCA cycle. This pathway is active under glucose limitation in slow-growing *E. coli* cultures in order to decouple catabolism from NADPH formation (Fischer and Sauer 2003).

Interestingly, the TCA cycle, the glyoxylate shunt and the acetate scavenging pathways are coregulated at the transcriptional and posttranslational levels. IclR is a specific repressor of the *aceBAK* operon which is regulated by glyoxylate and pyruvate (Sunnarborg et al. 1990; Lorca et al. 2007) and mutations affecting *iclR* indirectly affect *acs* expression (Shin et al. 1997; Kumari et al. 2000a). Moreover, the activity of isocitrate dehydrogenase, the TCA cycle enzyme which competes with isocitrate lyase for its substrate, is inhibited by the specific kinase activity of a bifunctional phosphatase/kinase protein (AceK), encoded by the *aceBAK* operon (Fig. 2) (Chung et al. 1988; Walsh and Koshland 1984). Additionally, isocitrate lyase is extensively acetylated at several lysine residues, which results in partial inhibition of its activity (Castaño-Cerezo et al. 2014).

## What bottlenecks the pyruvate node? Central carbon metabolism is regulated by global transcription factors

As early suggested, the unbalance between carbon source catabolism and cellular respiration could lead to the formation of a bottleneck at the pyruvate node and the accumulation of acetyl-CoA (Han et al. 1992; Varma and Palsson 1994).

Several global regulators control carbon metabolism, with a simultaneous impact on carbon metabolism balance, respiration, and efficiency of growth. The predominant regulators are the catabolite repressor activator (Cra or FruR), the cAMP receptor protein (CRP), and the aerobic respiration control protein (ArcA), which regulate the expression of glycolysis, gluconeogenesis, pyruvate dehydrogenase complex, acetate metabolism, TCA cycle, and glyoxylate shunt genes (Li et al. 2014; Perrenoud and Sauer 2005; Shalel-Levanon et al. 2005). These global regulators have opposing effects on the expression of metabolic pathway genes, but dominant effects are observed depending on the growth conditions. For instance, Li et al. determined that ArcA is the dominating transcription factor under nutrient excess conditions (Li et al. 2014).

ArcB is the membrane kinase sensor component of the system; the phosphorylated form of ArcA (ArcA-P) is a multimeric DNA-binding protein. The ArcAB system is controlled by the pool of membrane quinone electron carriers. The redox state of quinones reflects the activity of the respiration electron transport chain; limitations in the reoxidation of quinones by terminal oxidases and/or high rates of NAD(P)H generation shift the equilibrium to reduced quinones (Li et al. 2014). A high level of reduced quinones (quinols) leads to ArcA phosphorylation (Alvarez et al. 2013; Georgellis et al. 2001) and the resultant regulation of several operons. Among them, the genes encoding the enzymes from the TCA cycle, the glyoxylate shunt, and the pathway for fatty acid degradation are repressed by ArcA, while a few genes involved in fermentation are activated (Karp et al. 2014; Perrenoud and Sauer 2005; Shalel-Levanon et al. 2005). The repression of the TCA cycle genes by ArcAB is partially responsible for the formation of a bottleneck at the pyruvate node which ultimately favors the acetate overflow (Li et al. 2014).

## Transcriptional regulation of acetate scavenging: carbon catabolite repression

The expression of acetyl-CoA synthetase gene (*acs*) is tightly regulated. This gene is part of the *acs-yfcH-actP* operon. The *actP* gene encodes for an acetate membrane transporter (Gimenez et al. 2003) while the function of the membrane protein encoded by *yjcH* remains unknown. This operon is upregulated by carbon limitation, low oxygen pressure, increased flux through acetate-associated pathways (Kumari et al. 2000a), and during growth on acetate (Brown et al. 1977). Transcription is $\sigma^{70}$-dependent and repressed by $\sigma^{S}$ (Kumari et al. 2000b), and it is positively regulated by cAMP-CRP (Kumari et al. 2000a) and CsrA (Fig. 2) (Wei et al. 2000).

This pathway operates in the absence (or limitation) of PTS sugars, therefore permitting acetate scavenging. Consequently, the expression of this system is high in carbon-limited stationary phase of batch cultures, allowing for the consumption of the acetate accumulated during fast growth on glucose. Its activity is even more relevant in chemostat cultures, where the expression of the *acs-yfcH-actP* operon depends on the growth rate. In chemostat cultures, cAMP production is a function of the dilution rate. cAMP production levels are high at low dilution rate, and several CRP-dependent genes involved in increasing metabolic efficiency upon carbon-limiting conditions are upregulated, including the acetate uptake system, which prevents acetate overflow (Valgepea et al. 2010). Several authors have described the upregulation of *acs* and other genes in chemostat cultures of *E. coli* (Vemuri et al. 2006), although until very recently, this has not been recognized as a determinant fact for the acetate overflow phenomenon (Renilla et al. 2012; Valgepea et al. 2010). At high growth rate, the expression of this operon is limited by low stability of transcripts (Esquerré et al. 2014).

## Regulation of acetate metabolism by protein acetylation

Acetate metabolism and protein acetylation are intimately connected. Protein acetylation is a reversible posttranslational modification which is frequent in metabolic enzymes and may affect their function, contributing to global regulation of metabolism. It was first described in bacteria in *Salmonella* (Starai et al. 2004), although it is currently known to be widespread in prokaryotes (Bernal et al. 2014; Wolfe 2016).

Acetylation of proteins may be enzymatic, catalyzed by protein acetyl-transferases, or nonenzymatic, due to the inherent chemical reactivity of acylating compounds (such as acyl-CoAs and acyl-phosphates) which may accumulate in the cells (Weinert et al. 2013). Protein acetyltransferases catalyze the transfer of an acetyl moiety from acetyl-CoA to the amino group of a lysine residue on the target protein. Prokaryotic protein acetyltransferases are rather specific and many belong to the Gcn5-like acetyltransferase family (Crosby et al. 2012; Gardner and Escalante-Semerena 2008; Lima et al. 2012; Nambi et al. 2010; Thao and Escalante-Semerena 2011), as in the case of PatZ from *E. coli* (Castaño-Cerezo et al. 2011; de Diego Puente et al. 2015). Nonenzymatic acetylation of proteins by acetyl-phosphate and acetyl-CoA seems to be the main mechanism of protein acetylation in *E. coli* (Castaño-Cerezo et al. 2014; Galdieri et al. 2014; Kosono et al. 2015; Kuhn et al. 2014; Weinert et al. 2013). Acetylation is reversed by deacetylases, which may be simple hydrolases or $NAD^+$-dependent deacetylases (also known as sirtuins). Two deacetylases have been identified in *E. coli*: the sirtuin CobB and YcgC, belonging to the serine hydrolase family (Castaño-Cerezo et al. 2014; Castaño-Cerezo et al. 2011; Hayden et al. 2013; Starai et al. 2002; Tu et al. 2015; Zhao et al. 2004).

Although this is an emerging research field and many questions remained to be answered, the effects of protein acetylation on acetate metabolism have been described. In *E. coli* K-12, acetate metabolism is regulated by the protein acetyltransferase PatZ and the sirtuin CobB (Castaño-Cerezo et al. 2011; Weinert et al. 2013). The activity of acetyl-CoA synthetase is inhibited by acetylation of a conserved lysine residue in the vicinity of the substrate binding pocket (Castaño-Cerezo et al. 2014; Starai et al. 2002). This acetylation is catalyzed by PatZ (de Diego Puente et al. 2015) and is regulated by carbon catabolite repression (Fig. 2) (Castaño-Cerezo et al. 2011). Both *patZ* and *acs* genes share transcriptional regulation by cAMP-CRP (Castaño-Cerezo et al. 2011; Hentchel et al. 2015). At high acetyl-CoA levels, PatZ is activated by autoacetylation and subsequent oligomerization to form an octamer (de Diego Puente et al. 2015). Acetylation of acetyl-CoA synthetase inhibits its activity, limiting acetyl-CoA synthesis. Double deletion of *arcA* and *patZ* and overexpression of *acs* decreased acetate overflow in *E. coli* K-12 by simultaneously avoiding the inhibition of Acs by acetylation and the transcriptional repression of the TCA cycle and the glyoxylate shunt during fast growth (Peebo et al. 2014). This is the first application of protein acetylation to engineer *E. coli* metabolism.

Substrate specificity of the sirtuin CobB is seemingly low, and its deacetylase activity is global, contributing to the deacetylation of a large number of either chemically or enzymatically modified lysines (AbouElfetouh et al. 2015; Castaño-Cerezo et al. 2014), a role which has also been proposed for human sirtuin Sirt3 (Weinert et al. 2015). The newly discovered deacetylase YcgC is active on the transcriptional regulator RutR (Tu et al. 2015) and its role on acetate metabolism is unknown.

The occurrence of protein acetylation is also highly dependent on bacterial growth conditions and many evidences support that this is a regulated event. Protein acetylation increases in the stationary phase (Zhang et al. 2009), under nitrogen-limiting conditions (Weinert et al. 2013) and with different carbon sources (Castaño-Cerezo et al. 2014; Kosono et al. 2015). Deletion of *patZ* leads to overacetylation of the bacterial proteome during growth on acetate (Castaño-Cerezo et al. 2014). Altogether, these evidences suggest that *E. coli* has evolved a complex mechanism to self-regulate the pool of acetylating agents (acetyl-CoA and acetyl-phosphate), limiting their accumulation in the cells to avoid extensive acetylation lesions on cellular proteins. PatZ has a homeostatic role, controlling acetyl-CoA synthetase activity to avoid the overaccumulation of acetyl-CoA (Castaño-Cerezo et al. 2014).

## Acetate as a carbon source: the glucose to acetate transition

Catabolite repression mechanisms in *E. coli* K-12 are strong: in the presence of glucose, the metabolism of alternative carbon sources is repressed, making glucose the preferred nutrient. Consequently, when *E. coli* K-12 grows on glucose-acetate mixtures, both substrates are consumed sequentially. This is not the case of all *E. coli* strains. For instance, catabolite repression is not as stringent in *E. coli* BL21 and both carbon sources are simultaneously consumed (Castaño-Cerezo et al. 2015; Waegeman et al. 2012).

The glucose-acetate transition, consisting of events preparing metabolism for acetate utilization, is triggered in *E. coli* K-12 by glucose exhaustion. No diauxic growth behavior is observed in the glucose to acetate transition, unlike with other substrates such as lactate; acetate is consumed without concomitant growth. Enjalbert and colleagues have recently identified the reason for this behavior (Enjalbert et al. 2015). These authors measured the expression of catabolism and anabolism genes after glucose exhaustion. They discovered that, while

the expression of genes for acetate consumption (such as *acs*) is upregulated after glucose depletion, the expression of the glyoxylate shunt genes, which are needed for acetate anabolism, is low (Fig. 2). This is consistent with the lack of growth during the acetate consumption phase. The upregulation of acetate anabolism requires significant exposure to acetate (Enjalbert et al. 2015). This study suggested that delayed expression of acetate anabolism may be an adaptation to the permanently changing environmental conditions in gut. When sugars are not available, acetate catabolism fulfills energy requirements, allowing the cells to rapidly resume growth on preferred substrates, namely on sugars, if they become available (Enjalbert et al. 2015).

## Acetate overflow is related to growth rate-dependent global rearrangements

Several studies have related growth rate and global changes in the proteome and, more specifically, the need for increased ribosomal proteins to sustain protein synthesis at fast growth (Hui et al. 2015; Scott et al. 2010; You et al. 2013). Growth is limited by the cell's translation capacity, and the proteomic cost of protein translation increases with growth rate. In the last years, two independent works have proposed that global proteome rearrangements occurring in *E. coli* in response to growth rate ultimately cause acetate overflow (Basan et al. 2015; Peebo et al. 2015). Altogether, these works demonstrate that protein expression levels in *E. coli* are a result of the fine-tuned compromise between metabolic benefit and protein expression cost, ensuring optimal growth is achieved (Basan et al. 2015; Peebo et al. 2015). To balance the additional cost devoted to protein translation (e.g., ribosomal, amino acid and nucleobase metabolism proteins, translation factors, etc.), cells have to economize resources at the expense of other processes, namely those related to energetic metabolism. In other words, cells invest a lower fraction of their translational capacity for the synthesis of TCA cycle, glycolysis, oxidative phosphorylation, and transport proteins. The proteome cost of fermentation is half of that of respiration, which explains the higher overflow of acetate at high growth rates (Basan et al. 2015). In fact, downregulated proteins involved in aerobic respiration are high-cost components of the proteome, such as the ATP synthase and the proton motive force generating NADH dehydrogenase I (Peebo et al. 2015).

As outlined from several studies, acetate overflow occurs when cells grow at a growth rate higher than a threshold value, independently of the system analyzed (Basan et al. 2015; Peebo et al. 2015; Renilla et al. 2012; Valgepea et al. 2010). A mathematical model described by Basan and colleagues accounting for proteome costs and metabolic energy yields predicted the acetate overflow need for a balanced, optimal growth (Basan et al. 2015). Acetate overflow is inherent to the metabolism of *E. coli* and depends on the rate of carbon influx and not on the nature of the carbon source fueling metabolism.

These works have relevant implications in the protein production field, known to be limited by acetate production. Their mathematical model predicts that the threshold growth rate for acetate overflow can be reduced provided that the burden caused by recombinant protein overexpression is sufficiently high (Basan et al. 2015). This was previously observed by Sandén and colleagues, who described that recombinant protein production and the levels of ribosomal RNA were higher in cultures growing at high rate, and that high protein production was achieved at the expense of a higher acetate overflow (Sandén et al. 2003).

The molecular mechanisms behind growth-rate-dependent proteome resource allocation remain unknown, and it is likely the result of several regulating processes overlapping. A phenomenon contributing to growth-rate-dependent regulation of metabolism is the higher turnover of messenger RNA (mRNA) at high growth rates. This phenomenon is partly explained by transcriptional upregulation of genes encoding the mRNA degradation machinery and explains the downregulation of acetyl-CoA synthetase and TCA cycle genes, among others (Esquerré et al. 2014). You and colleagues proposed that cAMP-CRP coordinated global proteomic responses to changes in growth rate in carbon-limited cultures (You et al. 2013). Whether acetate overflow is due to repression of acetyl-CoA synthetase at low cAMP-CRP concentration or limited respiratory capacity (or both) (Peebo et al. 2015), and if other regulators play a role (Basan et al. 2015) still remains as open question. However, the driving force is related to the increasing need to direct more translational capacity for translation-related proteins and away from catabolic ones (Peebo et al. 2015).

## Overcoming acetate overflow: metabolic engineering strategies

Understanding the acetate overflow phenomenon at the molecular level has driven research to design strategies to avoid it. Overcoming acetate overflow has the potential to increase bioprocess yields, since products derived from central aerobic metabolism pathways often compete with acetate producing pathways for glucose as substrate. The optimization of the metabolism of *E. coli* for the production of acetyl-CoA-derived products under anaerobic or semianaerobic conditions usually involves the deletion of its fermentation pathways: *ackA-pta*, *poxB*, *ldhA*, *frdABCD* (encoding the fumarate reductase complex), *adhE* (encoding alcohol/aldehyde dehydrogenase), and *pflB* (encoding pyruvate-formate lyase) (Krivoruchko et al. 2015).

Additionally, acetate accumulation in *E. coli* cultures has always been a major drawback in bioprocess optimization.

Since many decades, biotechnologists have applied several strategies to avoid growth inhibition due to acetate accumulation. First strategies focused on the bioprocess level, setting up dialysis cultures, fed-batch cultivation schemes and optimizing media composition. More recently, metabolic engineering strategies have been applied (Eiteman and Altman 2006; De Mey et al. 2007b). With the advances in metabolic engineering, the molecular biology toolbox has been widely used for engineering acetate overflow and the metabolism of acetyl-CoA. These strategies can be sorted into three major groups: (i) engineering of acetate metabolism pathways (deleting or modulating acetyl-CoA generating enzymes); (ii) engineering of central carbon metabolism pathways (in order to yield a more balanced production of acetyl-CoA or attaining an efficient channeling of acetyl-CoA into downstream pathways); and (iii) engineering metabolism through molecular regulators. This topic has been covered in detail in previous reviews (Eiteman and Altman 2006; Krivoruchko et al. 2015; De Mey et al. 2007b; Shiloach and Fass 2005). An overview of the more successful recent contributions is given in Table 1.

## Is acetate metabolism different across strains? Acetate metabolism-related genes are conserved among different *E. coli* strains

Many efforts have been done studying the genomic and metabolic diversity of *E. coli* strains (Alteri and Mobley 2012; Bergholz et al. 2007; Fabich et al. 2008). The broad number of applications and niche varieties of the different *E. coli* strains makes them interesting for evolutionary studies (Alteri and Mobley 2012). Differences between the genomes of *E. coli* strains may be significant. For instance, the genome size of the pathogenic *E. coli* O157:H7 is a 20 % bigger than that of *E. coli* MG1655 (Hayashi 2001). Despite these differences between nonpathogenic and pathogenic strains, bioinformatics has shown that the core genome of most of these strains is common (Monk et al. 2013; Touchon et al. 2009). Genome-scale modeling of the metabolism of *E. coli* strains revealed differences between nonpathogenic (commensal) and pathogenic strains, especially in the catabolic pathways of carbon sources, reflecting the different lifestyles, environments, and available resources for survival (Monk et al. 2013).

When specifically considering acetate metabolism genes, a high degree of conservation among strains is observed, and all have the ability to grow aerobically on acetate as the sole carbon source (Monk et al. 2013). Frequently, intraspecies phenotype variations are explained by small differences in gene sequences, including silent mutation shifting sequences to using rare codons, or polymorphisms affecting gene regulatory regions, such as promoter regions. Examples of these variations have been found in *E. coli* BL21 (Waegeman et al. 2011). Further studies are needed to complete our understanding of the metabolic diversity among strains.

There are evidences of the multiple *E. coli* phenotypes underlining different strain capabilities. In the following sections, we aim to dissect the major differences in the acetate metabolism and its regulation concerning some *E. coli* strains, from nonpathogenic strains such as K-12, BL21, and W to pathogenic strains such as O157:H7.

## Low acetate-producing *E. coli* strains of biotechnological relevance: the BL21 and W strains

### *E. coli BL21*

Acetate metabolism is different between the two more common strains in laboratories, K-12 (MG1655) and BL21. The former strain has a long track record as preferred host for the production of recombinant proteins (Kim et al. 2015; De Mey et al. 2007b; Waegeman et al. 2011). Moreover, higher robustness and better performance at high glucose concentrations makes BL21 the candidate of choice for processes requiring fast growth rates, high biomass yields, and low accumulation of by-products (Marisch et al. 2013).

Sequencing of the genome of *E. coli* B strains explained some of its more salient characteristics such as the lack of motility or the absence of proteases (Jeong et al. 2009; Studier et al. 2009). Motility is irrelevant for bioprocesses in stirred tanks, and its absence may be advantageous for performance due to increased energetic efficiency. However, genome sequence was not enough to explain its most salient metabolic feature. During many years, the BL21 strain was described as a low acetate producer strain, and the causes to this phenotype were searched in its central metabolism (Marisch et al. 2013; Phue et al. 2005; Shiloach et al. 2010; Shiloach et al. 1996; Son et al. 2011; Waegeman et al. 2011). Several acetate metabolism pathways are more active in *E. coli* BL21 compared with K-12, including the glyoxylate shunt, gluconeogenesis, the anaplerotic pathways, and the TCA cycle (Noronha et al. 2000; Phue et al. 2005; Phue and Shiloach 2004), which suggests a more efficient acetate metabolism. Although many of these pathways are under the control of the transcriptional regulator Cra (catabolite repression activator, FruR), deletion of *cra* almost had no effect on their expression. Surprisingly, deletion of *cra* affected the expression of the osmoprotectant producing *betABC* operon in K-12, but not in BL21. These differences between strains might contribute to the higher robustness of the BL21 strain upon exposure to stress (Son et al. 2011).

Li et al. suggested that ArcA-P (the phosphorylated form of the aerobic respiration control protein) dominates the transcriptional and proteomic profile of BL21 cells under nutrient

**Table 1** Metabolic engineering strategies to overcome acetate overflow

| | Target | Effect | Reference |
|---|---|---|---|
| Engineering of acetate metabolism pathways | Deletion of *ackA-pta* and *poxB* | Reduces acetate production at the expense of growth rate and accumulation of pyruvate, formate, and lactate. The consequences may be highly dependent on environmental conditions, especially affecting anaerobic growth. | Dittrich et al. (2005), Niu et al. (2014), Castaño-Cerezo et al. (2009), Hädicke et al. (2015) |
| | Overexpression of acetyl-CoA synthetase (*acs*) | Acs is an acetate-scavenging enzyme. Overexpression decreases acetate accumulation. | Lin et al. (2006). |
| Engineering of central carbon metabolism pathways | Fine tuning of the expression of glycolysis genes *pgi* and *eno*. | Decreasing the rate of glycolysis avoids acetate production. The mutation in *pgi* enhances the pentose phosphate pathway, also increasing nucleotide biosynthesis, which is advantageous for plasmid DNA production. | Usui et al. (2012), Gonçalves et al. (2013) |
| | Glucose transporters phosphotransferase system ($PTS^{Glc}$) and permeases. | $PTS^{Glc}$ (encoded by *ptsI, ptsH*, and *ptsG*) couples glucose import and phosphorylation. Inactivation of *ptsG* reduces glucose import and acetate secretion and increases recombinant protein production. | Escalante et al. (2012), Chou et al. (1994) |
| | Overexpression of sugar permeases | Overexpression of *galP* and other sugar importers enhances recombinant protein production in a *ptsG* mutant. | De Anda et al. (2006), Fuentes et al. (2013) |
| | Overexpression of *mlc* | Mlc controls the expression of genes encoding $PTS^{Glc}$. | Cho et al. (2005) |
| Engineering acetate metabolism regulation | Transcriptional regulators of glyoxylate shunt *iclR* and *fadR* | Upregulation of the glyoxylate pathway and decreased acetate production. | Contiero et al. (2000), Farmer and Liao (1997) |
| | Transcriptional regulator of the transition from aerobic to anaerobic conditions *arcA* | Deletion of *arcA* proved a synergistic effect with the deletion of glyoxylate shunt regulators and also increased recombinant protein yield. | Waegeman et al. (2011, 2012, 2013) |
| | sRNA *sgrS*. | *sgrS* reduces the level of *ptsG* mRNA, decreasing glucose uptake and acetate production. It is transcribed in BL21 and K-12 strains, although it responds differently to high glucose concentrations in both strains. The expression of *sgrS* in *E. coli* K-12 decreases acetate production, mimicking the BL21 strain phenotype. | Negrete et al. (2013) |
| | sRNA *gadY* | Constitutive expression of (acid stress-related) *gadY* reduces acetate overflow and improves strain performance at low pH. | Negrete and Shiloach (2015) |
| | Regulation of metabolism by protein acetylation | Differences in posttranslational regulation have also been used to engineer acetate overflow. Deletion of *patZ* in K-12 and BL21 leads to different phenotypes: while acetate production increased in K-12, it was totally suppressed in the BL21 strain. | Castaño-Cerezo et al. (2015) |
| | Regulation of acetyl-CoA synthetase by acetylation | Double deletion of *arcA* and *patZ* and overexpression of *acs* decreased acetate production. | Peebo et al. (2014) |
| | Use of an acetyl-phosphate biosensor to regulate gene expression | Optimization of lycopene production by regulating the expression of key enzymes of this bioprocess. | Farmer and Liao (2000) |

excess conditions. Repression of the TCA cycle genes by ArcA-P creates a bottleneck at the pyruvate node, making cells prone to carbon overflow, even in the low acetate-producing BL21 strain (Li et al. 2014). Double deletion of *arcA* and *iclR* in K-12 avoids the repression of the TCA cycle and the glyoxylate shunt genes during fast growth and abolishes the acetate-producing phenotype of the K-12 strain, rendering a BL21-like phenotype (Waegeman et al. 2011). These mutations had no effect on the BL21 strain (Waegeman et al. 2012).

Recent studies have demonstrated that the lower acetate production in BL21 is a side effect of lower catabolite

repression. The higher accumulation of cAMP in this strain (Marisch et al. 2013) leads to enhanced expression of cAMP-CRP-activated genes, including the acetate metabolism *acs-yjcH-actP* operon and its posttranslational regulator *patZ* (Castaño-Cerezo et al. 2015). Thus, in the BL21 strain, acetate is simultaneously produced and scavenged due to an active futile cycle formed by the Pta-AckA and Acs pathways, which avoids its accumulation in the medium (Castaño-Cerezo et al. 2015).

Altogether, the differences in the acetate metabolism between the BL21 and the K-12 *E. coli* strains result from several overlapping effects, which are still to be fully described.

### *E. coli W*

*E. coli* W has a high biotechnological potential due to its ability to grow on sucrose as carbon source, with concomitantly low acetate production (Lee and Chang 1993). Arifin and collaborators have analyzed acetate overflow in this strain (Arifin et al. 2014). In *E. coli* W, the expression of *acs* and *actP* is not repressed during growth on sucrose. Acetate is scavenged and oxidized in the TCA cycle, since the glyoxylate shunt is not functional. Altogether, this evidences the different phenotypes of *E. coli* strains as a result of differential regulation of metabolic pathways.

## Acetate metabolism in gut

Gut microbiota contributes to the degradation of many complex carbohydrates and plant polysaccharides which cannot be degraded by animal enzymes, including cellulose, xylan, resistant starch, and inulin. Colonic primary fermenters yield short-chain fatty acids (acetate, propionate, and butyrate), gases ($H_2$ and $CO_2$), and energy for microbial growth (Gill et al. 2006; Tremaroli and Bäckhed 2012). Butyrate is substrate for the colonic epithelium and acetate and propionate for peripheral tissues (Tremaroli and Bäckhed 2012). The composition of the gut microbiota and the metabolic interactions between species affect food digestion, the efficiency of energy harvest, and even fat deposition.

Acetate metabolism by *E. coli* has a relevant role in the establishment and maintenance of gut microbiota. Early after birth, *E. coli* and *Enterococcus* are the first bacteria to colonize the sterile gut of newborns. Acetate and other short-chain fatty acids are produced, lowering oxygen concentration and pH creating a favorable environment for the colonization by anaerobic acidophiles. Facultative microorganisms, such as *E. coli*, contribute to maintaining the adult human gut reduced environment required by strict anaerobes (Wolfe 2005).

Concentrations of organic acids vary between the ileum and colon (with higher levels in the former). SCFA constitute two thirds of the anions present in the colon (Wolfe 2005). In addition, their concentrations are affected by diet (starch fermentation yields mainly butyrate whereas pectin fermentation yields more acetate) and are highly dependent on time after ingestion, since they are rapidly adsorbed (Herold et al. 2009).

The mechanism of the colonization of mammalian's gut by *E. coli* is not understood; very little is known about the metabolism of nutrients that support the growth and persistence of microbiota (Chang et al. 2004). Presumably, *E. coli* cross feeds in gut on simple sugars and SCFAs produced by strict anaerobes (De Vuyst and Leroy 2011; Wolfe 2005). Acetate and other organic acids such as propionate and butyrate are a natural carbon source for intestinal bacterial populations. Moreover, these compounds are involved in a remarkable number of processes. In microbial communities, a role in pathogenicy and in the communication with human cells and with other bacteria from gut has been proposed (Canny and McCormick 2008; Louis et al. 2014; Shoaie et al. 2013). Acetate metabolites interfere with signaling pathways regulating biofilm formation (see below) and the metabolism of autoinducer-2 (AI2) yields acetyl-CoA, showing a link between acetate metabolism and microbial communication by quorum sensing (Marques et al. 2014).

## Acetate metabolism in pathogenic *E. coli* strains

Intestinal pathogenic *E. coli* strains belong to one of the following six categories: enteropathogenic *E. coli* (EPEC), enterohaemorrhagic *E. coli* (EHEC), enterotoxigenic *E. coli* (ETEC), enteroaggregative *E. coli* (EAEC), enteroinvasive *E. coli* (EIEC), and diffusely adherent *E. coli* (DAEC). Extraintestinal infections are most frequent in the urinary tract, caused by uropathogenic *E. coli* (UPEC) or due to sepsis/meningitis, caused by meningitis-associated *E. coli* (MNEC). Further pathotypes have been described in animals (Kaper et al. 2004). These pathotypes are frequently sorted into serotypes according to O (lipopolysaccharide) and H (flagellar) antigens.

Compared to the nonpathogenic *E. coli* K-12 strain, very little information is available on the metabolism of *E. coli* pathogenic strains. However, recent studies point out that acetate metabolism is a key for the lifestyle of pathogenic strains, and it is closely related to the regulation of genes needed for pathogenesis.

### Acetate overflow in enteropathogenic and uropathogenic *E. coli*

The enteropathogenic *E. coli* O157:H7 strain exhibits high tolerance towards acetate. It has been explained by increased D-lactate production, contributing to decreasing transmembrane pH gradient and preventing the accumulation of intracellular acetate to toxic levels (Diez-Gonzalez and Russell

1997). In 2008, a study compared carbon nutrition in the MG1655 and O157:H7 strains in mouse intestine (Fabich et al. 2008). They observed that both strains produced similar amounts of acetate, and they only consumed it after other carbohydrates were exhausted. Deletion of *adhE* gene, which encodes the aldehyde-alcohol dehydrogenase, increased acetate production in the O157:H7 strain (Beckham et al. 2014), as in the K-12 strain (Zhu et al. 2005). Even more interestingly, this deletion decreased the strain virulence concomitantly (Beckham et al. 2014).

In uropathogenic *E. coli*, acetate is produced during growth in urine and acetogenesis contributes to in vivo fitness, but acetate reuptake does not (Anfora et al. 2008; Subashchandrabose and Mobley 2015). In bladder infection, acetate is produced due to overflow metabolism and not mixed acid fermentation (Hagan et al. 2010). Gene expression profiling revealed that the *ackA-pta* operon was highly expressed, while genes related to acetate consumption such as *aceA* and *acs* were downregulated. Moreover, *pta* and *ackA* genes contribute to urovirulence in a murine model (Hagan et al. 2010).

Thus, although contradictory, data indicate that altering acetate metabolism affects both enteropathogenesis and uropathogenesis of *E. coli* isolates. Mechanisms are likely to be strain-specific, which suggest that the effects of acetate metabolism on pathogenic traits might be pleiotropic.

## Acetate contributes to regulating the expression of virulence-related genes in pathogenic *E. coli*

Short-chain fatty acids enhance the virulence of *E. coli* and other Enterobacteriaceae (Wolfe 2005). The concentration of short-chain fatty acids in the human gastrointestinal tract modulates the expression of virulence-related genes in Shiga-toxigenic *E. coli* strains (STEC). STEC cause food borne infections, which commence with diarrhea and can progress to hemorrhagic colitis and the hemolytic uremic syndrome. STEC are classified into two major groups in accordance with the presence or absence of the locus of enterocyte effacement (LEE), which is related to pathogenicity. LEE-positive strains attach on the intestinal epithelium, effacing lesions on it. LEE-negative strains can also be virulent due to alternative accessory virulence factors, particularly adherence-mediating molecules. One of various adhesins identified is the Iha protein, which is distributed among LEE-negative and LEE-positive STEC, as well as in UPEC. Herold et al. (2009) analyzed the effects of concentrations of short-chain fatty acids in a hypervirulent LEE-negative STEC O113:H21 strain and two LEE-positive STEC O157:H7 strains. The concentrations of short-chain fatty acids mimicked the conditions usually found in the ileum and colon, respectively. High concentrations typical of colonic conditions strongly induced *iha* transcription and expression of Iha protein in all three strains, while induction was not observed under low concentrations of short-chain fatty acids mimicking ileal conditions (Herold et al. 2009). These findings are consistent with the belief that the colon, rather than the ileum, is the preferred site of STEC colonization in humans (Paton and Paton 1998).

Many of the infectious diseases that are associated to *E. coli* pathotypes are exacerbated by the formation of biofilm, and several authors have demonstrated the link between biofilm formation and the acetate metabolites acetyl-CoA and acetyl-phosphate (Lynnes et al. 2013; Wolfe 2005). Biofilm formation is regulated by several two component signal transduction systems. In the last years, there are increasing evidences that some response regulators of two component signal systems can be activated by intermediates of acetate metabolism. This is the case of RcsB (Thao et al. 2010), CpxA, and UvrY (Ma and Wood 2011) which can be acetylated by acetyl-CoA, or OmpR, which can be phosphorylated by acetyl-phosphate (Shin and Park 1995). Triggering of signal transduction cascades by acetate metabolites connects central metabolism and several other cellular processes (Wolfe 2005).

## Acetate metabolism and pathogenicity in related bacteria: role of protein acetylation in the pathogenicity of *Salmonella*

Very recently, the role of protein acetylation in the pathogenicity of *Salmonella typhymurium* has been demonstrated. The protein acetyltransferase *pat* from *S. typhymurium* is critical for bacterial intestinal colonization and systemic infection. RNA sequencing revealed that the expression of *Salmonella* pathogenicity island 1 (SPI-1) is partially dependent on the acetylation of the transcriptional regulator HilD (Sang et al. 2016). Invasion of spleen by *Salmonella* is also negatively controlled by Pat in a HilD-dependent mechanism. Pat controls HilD posttranscriptionally, moderately repressing gene translation and reducing the stability of the protein (Hung et al. 2016). *Salmonella*'s PhoP transcription factor is acetylated or deacetylated in response to environmental factors such as low magnesium, acid stress, or phagocytosis, which may be utilized to adapt the microorganism's physiology to hostile environments (Ren et al. 2016; Ren et al. 2015).

Other examples of global transcriptional regulation exerted by protein acetylation/deacetylation have been described in prokaryotes. In *E. coli*, the acetylation of RcsB impedes the transcriptional activation of all flagellar genes and genes of anaerobic metabolism and sugar acid degradation which transcription depends on the FlhD/FlhC complex (Castaño-Cerezo et al. 2014). FlhD/FlhC also impacts colonization of the mouse intestine (Horne et al. 2009). Taken together, these reports suggest that there is a clear link between acetate metabolism, acetate metabolites (acetyl-CoA and acetyl-phosphate), and signaling pathways crucial for pathogenicity. In *Salmonella enterica*,

dynamics of proteome acetylation affect pathogenicity (Hung et al. 2016; Ren et al. 2016; Sang et al. 2016), although this field has not been explored yet in *E. coli*, posttranslational acetylation of proteins in these two species is very similar, and conserved roles are to be expected.

The acetate switch has also been related to pathogenicity in other species. Acetate scavenging by *Vibrio cholerae* disrupts insulin signaling and lipid metabolism of the host (Hang et al. 2014). The intracellular pathogen *Shigella flexneri* possesses an active acetate metabolism during infection. It converts the pyruvate produced by the host cell into acetate, thus redirecting carbon metabolism fluxes of the infected cell for its own profit. This last finding made the authors propose that new inhibitors for the acetate catabolism proteins phosphotransacetylase and acetate kinase (which are not present in eukaryotes) might help fighting these infections (Kentner et al. 2014).

## Conclusions and outlook

Acetate metabolism in *E. coli* is highly regulated. The concurrence of several overlying mechanisms of regulation, exerting their effects at the transcriptional, posttranscriptional, posttranslational, and enzyme activity levels evidence its core importance for the physiology of *E. coli*. Activated forms of acetate are highly reactive, and their accumulation is prevented in order to avoid acetylation of proteins. Unregulated acetylation/deacetylation of proteins constitutes a substrate (or futile) cycle, leading to energy dissipation, and thus, has to be avoided for the sake of metabolic efficiency. Acetate metabolism of *E. coli* has physiological functions for the establishment and maintenance of gut microbiota, and it also modulates the pathogenicity of certain strains. Moreover, the major consequences of acetate overflow are a reduced efficiency in the metabolism of carbon sources, which has its major reflect on reduced metabolic yields, and the accumulation of acetate itself in the growth medium, which is toxic to cells as a consequence of the acidification of cytoplasm.

Dealing with acetate overflow is not a simple task: more than 20 years of metabolic engineering efforts can prove it. Single overexpression and inactivation of genes has proven inefficient to deal with this problem, and the most recent works demonstrate that it is an intrinsic problem derived from global regulation of metabolism and the way the cells optimize and economize their resources. The advent of novel systems and synthetic biology approaches will be needed to find more successful strategies.

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**Compliance with ethical standards**

**Conflict of interest** The authors declare that they have no conflict of interest.

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