Trends in
Microbiology

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Review

Role of metabolism in uropathogenic *Escherichia coli*

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Uropathogenic *Escherichia coli* (UPEC) is responsible for more than 75% of urinary tract infections (UTIs) and has been studied extensively to better understand the molecular underpinnings of infection and pathogenesis. Although the macromolecular adaptations UPEC employs – including the expression of virulence factors, adhesion molecules, and iron-acquisition systems – are well described, the role that metabolism plays in enabling infection is still unclear. However, a growing body of literature shows that metabolic function can have a profound impact on which strains can colonize the urinary tract. The goal of this review is to critically appraise this emerging body of literature to better understand the role that nutritional selection plays in enabling urinary tract colonization and the progression of UTIs.

Highlights

Uropathogenic *Escherichia coli* (UPEC) is adapted to metabolize a range of urinary compounds including amino acids, nucleic acids, and diverse secondary metabolites.

UPEC exhibits unique metabolic phenotypes that enhance its ability to colonize the urinary tract.

During infection, UPEC adjusts its metabolism to adapt to particular microenvironments within the urinary tract, allowing it to live planktonically in the urine or within bladder urothelial cells.

Microbial metabolism as a contributor to UTI

UTI (see Glossary) afflicts approximately 150 million people worldwide every year [1,2]. Understanding the molecular mechanisms that enable these infections is critical as it may provide a path to clinical advances [3–5]. Microbial virulence factors – agents which are produced to aid in establishing or maintaining infections – have been studied extensively in **UPEC**, the most common UTI pathogen [2,6]. These efforts have uncovered a variety of macromolecular mechanisms that enable UPEC to act as a pathogen, including: (i) adhesins that help UPEC bind to the urinary tract, (ii) type 1 pili that enable invasion of the host's bladder urothelium, (iii) toxins that destroy host cells, and (iv) iron acquisition systems that allow UPEC to obtain essential nutrients like iron [7–12]. Although these macromolecular mechanisms are clearly important, they are not the only contributors to the colonization of the urinary tract. Microbial metabolic capacity is also directly linked to which microbes can colonize a given environmental niche, and an emerging body of literature suggests that host/microbe metabolic interactions may play a role in UTIs [12–16].

Understanding the role that metabolism plays in UTIs is complicated by the overlapping impacts of host and microbial metabolic activities and their interplay with immunity. Differentiating between these overlapping effects is challenging, but has become more tractable over the past decade due to advances in transcriptomics [17–24], genomics [22,25–42], and most recently in metabolomics [4,43,44], where advances in high-resolution liquid chromatography–mass spectrometry have enabled the precise assessments of both host and pathogen metabolism. These strategies have allowed researchers to systematically interrogate the role specific metabolic pathways play in microbial fitness and virulence. The vast majority of these studies utilize either *in vitro* or *in vivo* metabolic knockout growth assays, and therefore comprehensive metabolic knockout UPEC strains have been constructed to determine which pathways are essential or dispensable during UTI [22,25–42]. Herein, we systematically review this emerging literature to help elucidate the underlying metabolic mechanisms that allow *E. coli* to colonize the urinary tract and become a **uropathogen**.

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Microbial metabolism in the urinary tract

Human urine is a complex medium with a rich transect of organic molecules. However, most high-energy nutritional sources have been depleted by host metabolism. Notably, urine normally lacks the carbohydrates that are the preferred carbon source for many microbes. Thus, microbes that are well-adapted to using diverse amino acids, nucleic acids, and secondary catabolites as food sources have a significant competitive edge. The role that these pathways play during the colonization of the urinary tract has been investigated, mainly through the use of metabolic knockouts in *in vitro* and *in vivo* models (Figure 1, Figure S1 in the supplemental information online, and Tables 1–4).

Amino acid catabolism and biosynthesis

Despite human urine being notorious for its highly variable chemical composition, amino acids and small peptides are consistently available at relatively high levels, and thus they are reliable energy sources for UPEC in the urinary tract. The total urinary amino acid concentration is estimated to be 5–7 mM, with most amino acids present at concentrations between 0.01 and 1.5 mM [13,45–48]. *E. coli* can utilize around half of these amino acids as nitrogen sources and around half as carbon sources [13,49]. Numerous transporters for peptides and amino acids are upregulated in UPEC during *in vitro* growth in human urine [17,18,21,22,25], but to date, only L-serine catabolism has been unambiguously demonstrated to be essential through metabolic knockout studies in murine models. Serine is one of the more abundant amino acids in human urine with a concentration ranging from 0.1 to 0.5 mM [13,45–48]. UPEC can catabolize urinary L-serine into ammonium and pyruvate using L-serine dehydratases (encoded by *sdaA* and *sdaB*), and then utilize the resultant catabolites to satisfy both carbon and nitrogen demands [26,27,49,50]. D-serine was originally thought to be a signalling molecule modulating virulence gene expression, based on the hypercolonization phenotype exhibited by D-serine dehydratase (*dsdA*) mutants in mouse models [27,41]. However, this hypercolonization phenotype was later demonstrated to be attributable to an unexpected secondary mutation in *rpoS*, rather than *dsdA*, and the mutation of *dsdA* had little to no effect on fitness in mice and human urine [26]. Additionally, D-serine import (*cycA*, *dsdX*) was dispensable during murine infection [26].

Despite the abundance of amino acids in urine, UPEC still relies on select amino acid biosynthetic pathways. These requirements have been shown through a series of systematic studies investigating which amino acid auxotrophies affect UPEC growth in human urine [51]. Strains that were auxotrophic for arginine, glutamine, leucine, methionine, serine, phenylalanine, and proline had growth defects in urine [51]. Of these auxotrophs, the arginine auxotrophs and biosynthetic knockout mutants had significant growth defects *in vitro* and *in vivo* [25,38–40,51]. Notably, although carbamoyl-phosphate synthase (*carAB*) is an integral enzyme in both arginine and *de novo* pyrimidine biosynthesis, the addition of arginine fully restored the *in vitro* growth of *carA* mutants in human urine, suggesting that *carA* contributes to fitness due to its involvement in arginine, rather than pyrimidine, biosynthesis [39]. UPEC's dependency on arginine biosynthesis is unexpected given that arginine auxotrophy is common amongst other bacterial pathogens – like *Pseudomonas aeruginosa* and *Neisseria gonorrhoeae* [52,53] – but may be attributable to arginine's role in polyamine biosynthesis (see 'Polyamine production' section below) [52,54,55]. High concentrations of putrescine, agmatine, and trimethylamine were detected in the urine of UPEC-infected patients compared to healthy controls, and the microbial secretion of these polyamines has been confirmed by *in vitro* human urine cultures [4,44]. Besides polyamine production, arginine may also be catabolized into glutamate and succinate through the arginine succinyltransferase pathway (encoded by the *ast* operon), but many of the *ast* genes were significantly downregulated during UTI in humans and mice, indicating that arginine may not feed into this pathway [24].

Glossary

Entner–Doudoroff pathway: a bacterial metabolic pathway that catabolizes glucose into pyruvate, as an alternative to glycolysis.
Gluconeogenesis: a metabolic pathway that generates glucose from pyruvate.
Glycolysis: a metabolic pathway that catabolizes glucose into pyruvate in order to generate high-energy molecules such as ATP and NADH.
Intracellular bacterial community (IBC): bacterial colonies that form inside host cells. During UTI, UPEC forms IBCs within urothelial cells.
Pentose phosphate pathway (PPP): a metabolic pathway that generates ribose, reducing power in the form of NADPH and glycolytic intermediates.
Tricarboxylic acid (TCA) cycle: a metabolic cycle that generates high-energy molecules through the oxidation of acetyl-CoA.
Urinary tract infection (UTI): an infection in any part of the urinary tract as a result of the colonization of a uropathogen.
Uropathogen: a microorganism that can cause UTI.
Uropathogenic *Escherichia coli* (UPEC): a pathotype of *E. coli* that causes UTI.

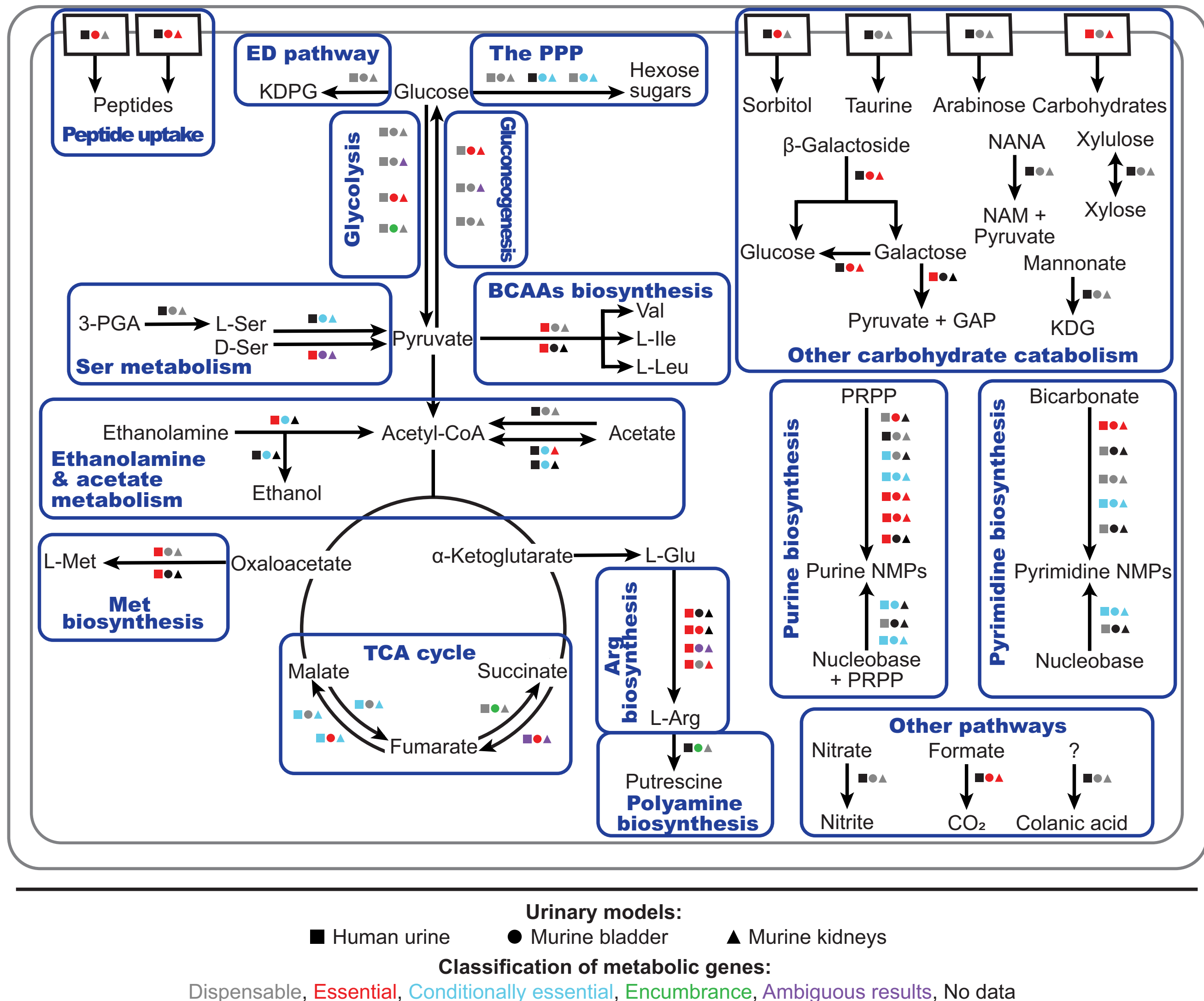

Figure 1. Metabolic pathways found to be essential and dispensable in uropathogenic *Escherichia coli* (UPEC) based on growth competition assays of metabolic knockouts in *in vitro* and *in vivo* models of the urinary tract. Metabolic knockout mutants were compiled from metabolic knockout studies using *in vitro* human urine cultures (square) and/or *in vivo* murine models of the bladder (circle) and kidneys (rectangle), as summarized in Tables 1–4. The network includes genes encoding metabolic enzymes (alongside reaction arrow) and transporters of metabolites (boxed); transcriptional regulators were omitted from this figure. The metabolic genes assessed were classified as either dispensable (grey), essential (red), only essential when coupled with specific proteins (blue), encumbers fitness and therefore improved fitness when disrupted (green), had mixed results (purple), or had no data in this model (black). These classifications were annotated onto the metabolic network. For a more detailed metabolic map containing gene names, please see Figure S1 in the supplemental information online. Abbreviations: Arg, arginine; BCAA, branched-chain amino acid; ED pathway, Entner–Doudoroff pathway; GAP, glyceraldehyde-3-phosphate; Ile, isoleucine; KDG, 2-dehydro-3-deoxy-D-gluconate; KDPG, 2-keto-3-deoxy-6-phosphogluconate; Leu, leucine; Met, methionine; NAM, N-acetylmannosamine; NANA, N-acetylneuraminate; NMP, nucleoside-monophosphate; 3-PGA, 3-phosphoglycerate; PPP, pentose phosphate pathway; PRPP, phosphoribosyl pyrophosphate; Ser, serine; TCA cycle, tricarboxylic acid cycle; Val, valine.

The biosynthesis of methionine and branched-chain amino acids (BCAAs) has also been shown to be essential during *in vitro* growth in human urine, as evidenced by the defective growth displayed by their respective biosynthetic knockouts [39,40]. However, these mutants were not outcompeted during experimental infection in mice [40]. UPEC utilizes these biosynthetic pathways during *in vitro* growth, because methionine is detectable in only around 41% of

human urine samples [45], and BCAAs are present at low concentrations of 0.01–0.05 mM [13,45–48]. By contrast, UPEC no longer requires these biosynthetic pathways during *in vivo* infection in mice, because UPEC is not limited to the scarce supply of nutrients in the urine and is capable of invading urothelial cells, utilizing host intracellular nutrients. In summary, arginine biosynthesis and L-serine catabolism have been determined to be vital for *in vivo* and *in vitro* growth. Other amino acids, like BCAAs and methionine, may need to be synthesized only during planktonic growth in urine.

Carbohydrate metabolism

One of the most shocking observations from the literature is that **glycolysis** appears to be dispensable in UPEC, even though *E. coli* is famous for being a carbohydrate-utilizing microorganism, and carbohydrates are present in urine at around 4 mM [13]. However, the carbohydrates in urine are not ideal nutrients for a variety of reasons. Firstly, glucose, the most preferred carbohydrate for supporting microbial growth, has a rather low concentration of around 0.29 mM in urine [13]. An exception to this is in diabetic patients, who characteristically exhibit excessive urinary glucose (glucosuria). Interestingly, though it is well established that diabetic patients are at increased risk for UTI and UTI complications [56–59], the precise role of glucosuria in perpetuating these negative outcomes remains unclear [60–64], but host immune dysfunction or increased UPEC adherence may contribute to the frequent occurrence of UTIs in diabetic patients [65,66]. Secondly, urothelial host cells may limit glucose availability by upregulating glucose import during UTI to either sequester it from pathogens or to capture it for their own energy use [67]. Thirdly, the majority of the carbohydrates present in urine are not optimal carbon sources for UPEC growth. The catabolic enzymes needed to metabolize these less favourable carbohydrates, including arabinose, sialic acid, hexuronate, and xylose, were found to be dispensable, suggesting that these carbohydrates may not utilized by UPEC [25]. Unexpectedly, some carbohydrate utilization genes were upregulated during *in vitro* and *in vivo* models; however, the gene expression data are often conflicting [18,21,22,24,25,30]. Fourthly, loss-of-function in essential glycolytic enzymes, including the rate-controlling phosphofructokinase 1 and 2 (*pfkA*, *pfkB*) and pyruvate kinase I and II (*pykF*, *pykA*), has minimal impact on the fitness of UPEC in both *in vivo* and *in vitro* models [35,36]. Strangely, single knockouts of *pykF* (the gene responsible for 80% of pyruvate kinase activity) cause defective growth, whereas double knockouts of *pykF* and *pykA* have normal growth [36]. The reason for the discrepancy between the single and double knockout is unclear, but it is evident that complete loss of pyruvate kinase activity is not detrimental to UPEC growth [30,36,68]. Unsurprisingly, other metabolic pathways closely linked to glycolysis are also dispensable, including the oxidative branch of the **pentose phosphate pathway** (**PPP**) and the **Entner–Doudoroff pathway**, as enzyme knockouts in either of these pathways grew normally both *in vitro* and *in vivo* [25,35]. In summary, these data suggest that, in urine, UPEC likely does not catabolize the available carbohydrates, preferring instead to obtain carbon from amino acids.

Although UPEC may have minimal requirement to catabolize sugars, it appears to maintain its gluconeogenic capacity. Evidence for this includes the observation that the rate-controlling step for **gluconeogenesis**, catalysed by phosphoenolpyruvate carboxykinase (*pckA*), was found to be essential *in vivo* [25,35]. Moreover, pathways downstream of gluconeogenesis, including the non-oxidative branch of the PPP, have been found to be essential. Confusingly, two enzymes that are shared between gluconeogenesis and glycolysis, triosephosphate isomerase (*tpiA*) and phosphoglucose isomerase (*pgi*), were largely found to be dispensable both *in vitro* and *in vivo* [25,35]. The reason for this is unclear; however, it may be attributable to the fact that carbon entering gluconeogenesis is diverted into the non-oxidative branches of the PPP to synthesize ribose, and thus, may not require these shared enzymes. Collectively, these findings are consistent with the observation that amino acids, not carbohydrates, serve as the primary carbon source for UPEC.

Tricarboxylic acid cycle

The **tricarboxylic acid (TCA) cycle** functions as the primary energy-generating pathway, serving as the link between carbon metabolism and oxidative phosphorylation, and it is the central pathway tied to the interconversion of many metabolites. This cycle can run either oxidatively or reductively, depending on the metabolic needs of the organism. In the urinary tract, the bladder is moderately oxygenated with urinary oxygen levels between 4% and 5.5% [18,69,70], which is sufficient to favour oxidative over reductive flux. *In vivo* knockout studies have shown that the oxidative enzymes fumarate hydratase class II and succinate dehydrogenase (*fumC*, *sdhB*) are essential, whereas reductive enzymes fumarate hydratase and fumarate reductase class I (*fumB*, *frdA*) appear to be dispensable [25,35,37]. These observations suggest that oxidative TCA flux is essential in UPEC.

Furthermore, electron donors generated by oxidative TCA may fuel the electron transport chain, in which multiple respiratory complexes are used to generate energy. Several of these complexes require iron–sulfur clusters to function [71], and this may explain the well-established importance of iron acquisition in UPEC, a topic that has been extensively reviewed elsewhere [7,8,11,72,73].

Nucleic acid biosynthesis

The rapid growth UPEC undergoes during infection depends on large pools of nucleotides for DNA replication. The current literature suggests that the mechanism UPEC uses to satisfy these demands depends on the stage of infection, with both the salvage and *de novo* biosynthetic pathways playing essential roles at different stages. For planktonic bacteria growing in urine, multiple *in vitro* studies have shown that knockouts for enzymes in UPEC's *de novo* purine biosynthesis have minimal impact on growth, whereas knockouts of the salvage pathway enzymes significantly disrupt growth [28,29,39,40]. Similar experiments on pyrimidine biosynthesis showed that UPEC needs one of these pathways to be intact, but surprisingly, losing either the *de novo* or salvage pathways was evidently equivalent [39,74]. Collectively, these data indicated that the approximate 200 μM of nucleobases and 20 μM of nucleosides present in human urine are sufficient to support UPEC's nucleotide demands through the salvage pathways during planktonic growth [13].

These nucleotide biosynthetic requirements appear to change when experiments are conducted using *in vivo* murine infection models: the knockout of amidophosphoribosyltransferase (*purF*), the rate-limiting enzyme in purine *de novo* biosynthesis, has a significant impact on growth [28]. The discrepancy between the *in vitro* and *in vivo* models in this context may be attributable to the formation of **intracellular bacterial communities (IBCs)**, an invasive growth form of *E. coli* that is common in UPEC infections. Within this intracellular milieu, UPEC may not have access to sufficient purines to replicate, necessitating their *de novo* biosynthesis. Meanwhile, pyrimidine biosynthesis evidently follows a different pattern where, similar to the results observed *in vitro*, either the *de novo* or salvage pathway can support growth [39,74]. Why the selective pressures affecting pyrimidine and purine biosynthesis do not follow the same patterns for planktonic growth and in IBCs is unclear.

Polyamine production

UPEC produces a range of diamines and polyamines, including putrescine, agmatine, cadaverine, and trimethylamine (herein collectively referred to as 'polyamines') [4,44,75]. Polyamines have been implicated in the physiological stress response, biofilm formation, and evasion of host defences [54,76,77]. UPEC may produce agmatine, cadaverine, putrescine, and γ-aminobutyric acid to protect against the acidic conditions in the urine [78]. This acid resistance may be enabled via four amino acid decarboxylase acid resistance systems present in *E. coli* [78]. Each of these systems transports H^+ out of the cytosol by importing an extracellular amino acid, decarboxylating

the amino acid using a proton-consuming reaction mechanism, and secreting the protonated/decarboxylated product back into the extracellular space [78]. Specifically, the lysine-dependent acid-resistance system encoded by the *cad* operon was found to be essential for UPEC growth, since *cad* knockouts were less fit and more sensitive to acidic and nitrosative stress [77]. Although our understanding of the biochemical underpinnings of polyamine production is still evolving, it is clear that UPEC is a prolific polyamine producer [4,44].

Ethanolamine and acetate metabolism

Ethanolamine is present in human urine at around 0.4–0.7 mM and can be a source of nitrogen for UPEC [32]. UPEC has been shown to consume ethanolamine when grown in artificial urine, and the disruption of ethanolamine catabolic genes (*eut* operon) reduces growth in both *in vitro* and *in vivo* models [32,33]. The *eut* operon mediates the catabolism of ethanolamine into ammonia and acetaldehyde [32]. The ammonia from this reaction is then utilized as a nitrogen source, while the acetaldehyde can be further processed into acetyl-CoA or acetate [32].

The Pta-AckA pathway is known to control the bidirectional acetate flux between *E. coli* and its environment [79,80]. This bidirectional pathway functions to either produce and secrete acetate produced within the cell or import and utilize extracellular sources [79,80]. Enzymes in the Pta-AckA pathway (*pta*, *ackA*) were highly upregulated *in vitro* and were found to be essential *in vivo*, whereas the alternative acetate assimilation gene (*acs*) was strongly downregulated *in vitro* and was found to be dispensable *in vivo* [17,21,24,42]. When grown in artificial urine, UPEC initially secreted acetate and then later consumed extracellular acetate [32]. These results suggest that UPEC regulates acetate flux primarily through the Pta-AckA pathway and may readily switch between acetate production and consumption depending on the availability of acetate and acetogenic compounds in its environment [79,80]. UPEC may regulate acetate metabolism to maintain functional levels of acetyl phosphate, an intermediate in the Pta-AckA pathway, and a major phosphate and acetyl donor involved in many two-component regulatory systems that mediate stress responses and virulence factor expression [42,80–83]. In summary, controlling acetate metabolism is essential in UPEC, possibly for maintaining appropriate acetyl phosphate levels vital to the regulation of many cellular processes.

Current metabolic model of UPEC

Based on the current literature, it appears that UPEC primarily relies on the catabolism of amino acids (most notably L-serine), rather than carbohydrates to fuel other downstream pathways, including gluconeogenesis and the TCA cycle. UPEC uses the oxidative TCA to generate energy and synthesize essential amino acids like arginine (the primary precursor of polyamines – molecules involved in resistance against acidic and nitrosative stress) and may either import or synthesize other amino acids depending on its current urinary environment. UPEC may utilize gluconeogenesis and the oxidative branch of the PPP to generate ribose for nucleotide synthesis. It appears that UPEC also utilizes extracellular ethanolamine as a source of nitrogen and acetate, and the acetate may be converted to acetyl phosphate, which regulates many cellular functions. These observations are summarized as a diagram (Figure 2, Key figure) and elaborated upon in metabolic network depicting the results from knockout studies (Figure 1 and Figure S1). All metabolic reactions included in this network are described in further detail in Tables 1–4.

Stages of infection and their metabolic requirements

To colonize the urinary tract, UPEC is exposed to multiple metabolic selective events as it is forced to adjust its metabolism to survive in drastically different microenvironments. UPEC

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arose from enteric bacteria shed from faeces that have travelled up the urethra to the bladder [13,15]. During the colonization of the bladder, UPEC has to survive and rapidly replicate, with studies estimating a doubling time of 22 min for human UTIs, based on assessments of both planktonic growth in human urine and intracellular growth during *in vivo* murine UTIs [84]. This rapid growth rate contributes to UPEC's spread and persistence in the urinary tract. However, the chemical composition of human urine and urothelial cytosol can differ drastically, and UPEC has seemingly evolved the winning combination of metabolic adaptions that enable it to succeed in these two disparate microenvironments within the urinary tract.

An example of this differential metabolic regulation is UPEC's use of the *de novo* biosynthetic or salvage pathways to produce purines depending on whether it is growing planktonically or within host urothelial cells, and either pathway to acquire pyrimidines irrespective of its microenvironment (see earlier section 'Nucleic acid biosynthesis'). Similarly, UPEC catabolizes sorbitol and β-galactosides specifically within IBCs [85–88]. The sorbitol and β-galactoside transport and catabolic genes are significantly upregulated and found to be essential specifically in IBCs [14,31]. Sorbitol is imported and concentrated within host bladder epithelial cells to regulate osmotic tension and maintain their cell volume [31]. UPEC may metabolize its host's intracellular supply of sorbitol to generate glucose [14,31]. Similarly, UPEC may take up and cleave intracellular β-galactosides to produce galactose, which can be converted to glucose or metabolized through the DeLey-Doudoroff pathway [31,39].

Current methodology and emerging frontiers

Broadly speaking, data obtained from the two most common experimental models used to study the effect of UPEC metabolic knockouts, *in vitro* culture in human urine and *in vivo* murine models of UTI, do not always correlate. This may be attributable to interspecies differences in the urinary chemical composition and urinary tract physiology between humans and mice, which undoubtedly influences the metabolic activities of UPEC in these different experimental milieus [89–91]. It also may be attributable to the fact that these two models respectively represent different stages of colonization and infection, with urine growth experiments emulating only the planktonic growth stage while *in vivo* murine model experiments consider the entire infection process, including IBC growth. It is therefore unsurprising that there are discrepancies between the results obtained between these two model systems. Comparing the differences in the growth of a given metabolic knockout between these two models provides insight into the role of microbial metabolism at different stages of infection, with experiments in human urine pinpointing metabolic processes important for planktonic growth and murine UTI models pinpointing processes important in overall progression of infection in the mammalian urinary tract.

Future experiments are required to determine the relative importance of each of these UPEC metabolic processes at each stage of infection, and their relative contribution to its overall uropathogenicity in the context of human health (see Outstanding questions). This may require *in vitro* human bladder cell-based modelling systems, where both extracellular and intracellular stages of infection can be assessed separately. Examples include cell monolayers, tissue and organ cultures, and 3D organoids [89,92]. However, there are many limitations to mimicking bladder physiology. For instance, current models are rarely exposed to urine for long periods of time, unlike in the native bladder microenvironment, making it challenging to study uropathogen metabolism extracellularly [89]. Hence, future studies should focus on developing and improving authentic *in vitro* cell-based model systems for studying uropathogen metabolism during intracellular and extracellular stages of infection.

Outstanding questions

Are there other unidentified metabolic pathways that are utilized by UPEC to colonize the urinary tract?

How do UPEC's metabolic adaptations enable it to cause UTI, and are these adaptations present in other uropathogens?

What regulates the metabolic changes that UPEC undergoes when transitioning from the planktonic stage in urine and the IBC stage in host urothelial cells?

Which new experimental models can accurately decipher the interplay between host and pathogen metabolism during both extracellular and intracellular stages of infection?

How can knowledge of uropathogen metabolism be applied to the development of novel, innovative improvements in the clinical management of UTIs?

Key figure

Model of uropathogenic *Escherichia coli (*UPEC) metabolism during urinary tract infection (UTI)

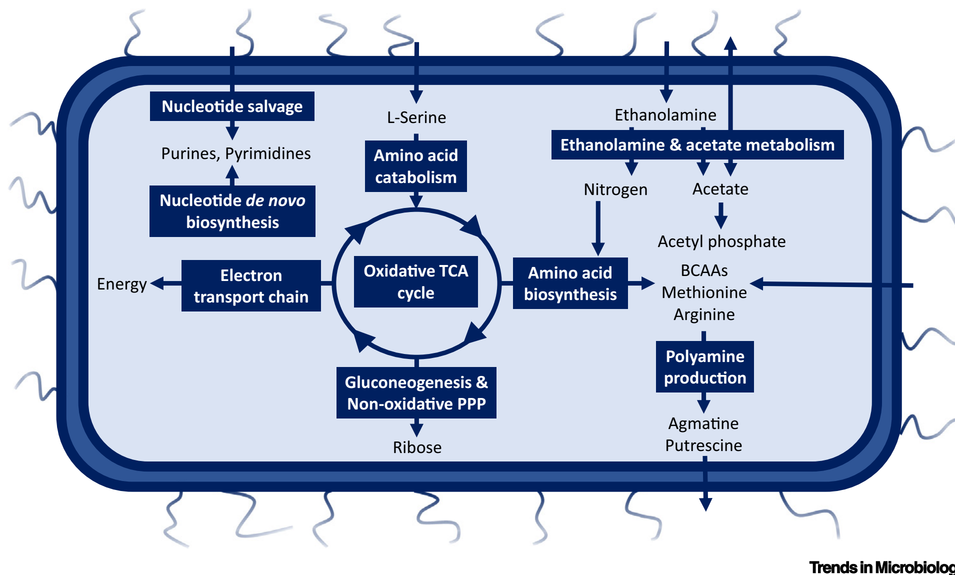

Figure 2. Based on the current literature, UPEC requires all the illustrated metabolic pathways to successfully colonize the urinary tract. UPEC takes up amino acids like serine to fuel the tricarboxylic acid (TCA) cycle, which generates many precursors that can feed into multiple downstream pathways, including the electron transport chain, gluconeogenesis, the non-oxidative branch of the pentose phosphate pathway (PPP), and amino acid biosynthesis and import. UPEC may take up ethanolamine as a source of nitrogen to fuel amino acid biosynthesis, and a source of acetate to produce acetyl phosphate. The amino acids arginine and lysine (not shown) are the precursors to many polyamines. UPEC may also utilize either the salvage or *de novo* biosynthetic pathway to generate nucleotides. Abbreviation: BCAA, branched-chain amino acid.

Concluding remarks and future perspectives

The emerging literature demonstrates that microbial metabolism plays a vital role in UTIs, and this is especially evident in the catabolism and biosynthesis of specific amino acids, the oxidative TCA cycle, purine and pyrimidine biosynthesis, ethanolamine utilization, acetyl phosphate production, and sorbitol and β-galactoside catabolism. However, the metabolic model of UPEC is far from complete and its overall biological and clinical implications have yet to be uncovered (see Outstanding questions), due in part to challenges associated with piecing together the results from numerous different isolated experiments to form a single robust metabolic model. Important distinctions between planktonic and intracellular metabolism may explain some conflicting studies. Future work should therefore consider the role of a given metabolic process in a specific microenvironment. A more comprehensive understanding of the inner metabolic workings of UPEC may reveal novel targets for developing clinical technologies that improve the management of UTIs.

Table 1. Metabolic genes involved in central carbon metabolism and their contribution to UTI

Metabolic gene description[a]			Results from knockout growth experiments[b]						Results from transcriptomics analyses[c]			
Gene	Metabolic function	Reaction catalysed	Mouse bladder[d]	Mouse kidneys[d]	Mouse urine[d]	Human urine[d]	*E. coli* Strain	Refs	*In vivo* Human urine[e]	*In vitro* Human urine[e]	*In vivo* Mouse urine[e]	Refs
Glycolysis												
ptsG	Glucose-specific IIBC component in the PTS for glucose transport	N/A							↑U			[21]
pgi	Phosphoglucose isomerase	D-glucose 6-phosphate ↔ D-fructose 6-phosphate	+ +	+ +		+ +	CFT073	[25,35]	↓U			[23]
pfkA	6-phosphofructokinase 1	D-fructose 6-phosphate + ATP ➔ D-fructose 1,6-bisphosphate + ADP + H^+	+ +	+ +		+	CFT073	[35,36]				
pfkB	6-phosphofructokinase 2	Same as *pfkA*	+	+		+	CFT073	[36]	↓U			[23]
fbaB	Fructose-bisphosphate aldolase class I	D-fructofuranose 1,6-bisphosphate ↔ glycerone phosphate + D-glyceraldehyde 3-phosphate							↓U			[21]
tpiA	Triosephosphate isomerase	Dihydroxyacetone phosphate ↔ D-glyceraldehyde-3-phosphate	+ +	– +		+	CFT073	[25,35]	↓U	↑M		[17,25]
gpmM	Phosphoglycerate mutase III	2-phosphoglycerate ↔ 3-phosphoglycerate							↑U			[21]
pykA	Pyruvate kinase II	Phosphoenolpyruvate + ADP + H^+ ➔ pyruvate + ATP	++ ++	+ +		+	CFT073	[35,36]				
pykF	Pyruvate kinase I	Same as *pykA*	–	–		+	CFT073	[36]	↑U			[21]
pfkAB	6-phosphofructokinase isozymes See above for *pfkA*, *pfkB*		++	+		+	CFT073	[36]				
pykAF	Pyruvate kinase isozymes See above for *pfkA*, *pfkB*		+	+		+	CFT073	[36]				
Gluconeogenesis												
pckA	Phosphoenolpyruvate carboxykinase	Oxaloacetate + ATP ➔ phosphoenolpyruvate + ADP + CO_2	– –	– –		+	CFT073	[25,35]	↓U			[17]
tpiA	See above for *tpiA* in the 'Glycolysis' section											
fbaB	See above for *fbaB* in the 'Glycolysis' section											
pgi	See above for *pgi* in the 'Glycolysis' section											
Pentose phosphate pathway (PPP)												
gntK	D-gluconate kinase in D-gluconate catabolism feeding into oxidative PPP	D-gluconate + ATP ➔ D-gluconate 6-phosphate + ADP + H^+							↑M, ↑U ↑U		↑M, ↑U	[21,24]
gnd	6-phosphogluconate dehydrogenase in oxidative PPP	6-phospho-D-gluconate + $NADP^+$ ➔ CO_2 + D-ribulose 5-phosphate +	+ +	+ +		+	CFT073	[25,35]				

(continued on next page)

Table 1. (continued)

Metabolic gene description[a]			Results from knockout growth experiments[b]						Results from transcriptomics analyses[c]			
Gene	Metabolic function	Reaction catalysed	Mouse bladder[d]	Mouse kidneys[d]	Mouse urine[d]	Human urine[d]	*E. coli* Strain	Refs	*In vivo* Human urine[e]	*In vitro* Human urine[e]	*In vivo* Mouse urine[e]	Refs
		NADPH										
talA	Transaldolase in nonoxidative PPP	D-glyceraldehyde 3-phosphate + D-sedoheptulose 7-phosphate ➔ β-D-fructose 6-phosphate + D-erythrose 4-phosphate	+ ++	+ +		+	CFT073	[25,35]	↓U ↓U ↓U ↓M	↑M	↓U	[17,21, 23–25]
talB	Transaldolase in nonoxidative PPP	D-glyceraldehyde 3-phosphate + D-sedoheptulose 7-phosphate ➔ β-D-fructose 6-phosphate + D-erythrose 4-phosphate	+	+			CFT073	[35]				
talAB	Transaldolase isozymes involved in nonoxidative PPP See above for *talA*, *talB*		–	–			CFT073	[35]				
Entner–Doudoroff pathway												
edd	6-phosphogluconate dehydratase	6-phospho-D-gluconate ➔ 2-keto-3-deoxy-6-phosphogluconate + H_2O	+ +	+ +		+	CFT073	[25,35]				
Bridging reaction and tricarboxylic acid (TCA) cycle												
aceE	Pyruvate dehydrogenase E1 component of the pyruvate dehydrogenase complex in the bridging reaction	Pyruvate + [protein]-N^6-lipoyl-L-lysine + H^+ ➔ [protein]-N^6-(S^8-acetyldihydrolipoyl)-L-lysine residue + CO_2							↑T ↑M			[17,23]
lpdA	Dihydrolipoyl dehydrogenase E3 component of the pyruvate dehydrogenase complex in the bridging reaction	NAD^+ + N^6-dihydrolipoyl-L-lysyl-[protein] ➔ NADH + N^6-lipoyl-L-lysyl-[protein]							↑T ↑M			[17,23]
citT	Citrate and succinate transporter	N/A							↓U			[21]
gltA	Citrate synthase in oxidative TCA	Oxaloacetate + acetyl-CoA + H_2O ➔ citrate + CoA + H^+							↓M, ↓U ↓U		↓M, ↓U	[21,24]
acnA	Aconitate hydratase A in TCA	Citrate ↔ isocitrate							↓U ↓M			[17,23]
acnB	Aconitate hydratase B in TCA	Same as *acnA*							↓M		↓M	[24]
kgtP	α-ketoglutarate permease for α-ketoglutarate transport	N/A							↓U ↓U		↓U	[17,24]
sdhB	Succinate dehydrogenase iron-sulfur subunit in oxidative TCA	Succinate + quinone ➔ fumarate + quinol	– –	– +		+ –	CFT073	[25,35,37]	↓M ↓U		↓M	[17,24]
fumA[f]	Fumarate hydratase class I in oxidative TCA	Fumarate + H_2O ➔ malate	+	+		+	CFT073	[37]	↓U			[17]

fumB	Fumarate hydratase class I in reductive TCA	Malate ➔ fumarate + H_2O	+	+		+	CFT073	[37]				
fumC	Fumarate hydratase class II in oxidative TCA	Same as *fumA*	– –	+ +		+	CFT073	[35,37]	↓U ↓M		↓U	[23,24]
frdA	Fumarate reductase subunit in reductive TCA	Fumarate + quinol ➔ succinate + quinone	++	+		+	CFT073	[35,37]	↓U			[21]
frdB	Fumarate reductase subunit in reductive TCA	Same as *frdA*							↓U			[21]
frdC	Fumarate reductase subunit in reductive TCA	Same as *frdA*							↓U			[21]
mdh	Malate dehydrogenase in TCA	Malate + NAD^+ ↔ oxaloacetate + NADH + H^+							↓U			[21]
sdhB *frdA*	Enzymes in oxidative and reductive TCA See above for *sdhB*, *frdA*		++	+		–	CFT073	[37]				
fumAB	Fumarate hydratase class I isozymes in oxidative/reductive TCA See above for *fumA*, *fumB*		++	+		+	CFT073	[37]				
fumAC	Fumarate hydratase isozymes in oxidative TCA See above for *fumA*, *fumC*		–	–		+	CFT073	[37]				
fumBC	Fumarate hydratase isozymes in oxidative/reductive TCA See above for *fumB*, *fumC*		–	–		+	CFT073	[37]				
fumABC	Fumarate hydratase isozymes in oxidative/reductive TCA See above for *fumA*, *fumB*, *fumC*		–	–		–	CFT073	[37]				
Glyoxylate shunt												
aceA	Isocitrate lyase	Isocitrate ➔ glyoxylate + succinate							↓U ↓U ↓M			[17,21,23]
aceB	Malate synthase A	Acetyl-CoA + glyoxylate + H_2O ➔ malate + CoA + H^+							↓U ↓U ↓M			[17,21,23]
Electron transport chain												
cyoD	Cytochrome bo_3 subunit 4	2 $ubiquinol_{[inner\ membrane]}$ + 8 H^+ + O_2 ➔ 2 $ubiquinone_{[inner\ membrane]}$ + 8 $H^+_{[periplasm]}$ + 2 H_2O								↑M		[18]
cyoE	Heme O synthase in cytochrome bo_3 complex	Protoheme + farnesyl diphosphate + H_2O ➔ ferroheme *o* + PP_i								↑M	↑M	[18]
cydA	Cytochrome *bd*-I subunit in microaerobic respiration	2 $ubiquinol_{[inner\ membrane]}$ + 4 H^+ + O_2 ➔ 2 $ubiquinone_{[inner\ membrane]}$ + 4 $H^+_{[periplasm]}$ + 2 H_2O							↑T ↑M			[17,23]
poxB	Pyruvate oxidase in pyruvate to cytochrome *bd* electron transfer	Pyruvate + $ubiquinone_{[inner\ membrane]}$ + H_2O ➔ CO_2 + acetate + $ubiquinol_{[inner\ membrane]}$ Pyruvate + acetaldehyde + H^+ ➔ acetoin + CO_2							↓U ↓U ↓M		↓U	[21,23,24]

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Declaration of interests

No interests are declared.

Supplemental information

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Notes to Table 1:

[a]Abbreviations of metabolites and cofactors include: ABC, ATP-binding cassette; ACP, acyl-carrier protein; ADP, adenosine diphosphate; AICAR, 5-aminoimidazole carboxamide ribonucleotide; AIR, 5′-phosphoribosyl-5-aminoimidazole; AMP, adenosine monophosphate; ATP, adenosine triphosphate; CAIR, 5′-phosphoribosyl-4-carboxy-5-aminoimidazole; N5-CAIR, N5-carboxyaminoimidazole ribonucleotide; (d)CMP, (deoxy)cytidine monophosphate; CoA, coenzyme A; FAICAR, 5-formamidoimidazole-4-carboxamide ribotide; FGAM, 5′-phosphoribosylformylglycinamidine; FGAR, formyl-phosphoribosylglycinamide; N10-formyl-THF, 10-formyltetrahydrofolate; GABA, γ-aminobutyric acid; GAR, glycineamide ribonucleotide; GDP, guanosine diphosphate; GMP, guanosine monophosphate; GTP, guanosine triphosphate; IMP, inosine monophosphate; 5-MTHF, 5-methyltetrahydrofolate; NAD^+, nicotinamide adenine dinucleotide; NADH, nicotinamide adenine dinucleotide hydrogen; $NADP^+$, nicotinamide adenine dinucleotide phosphate; NADPH, nicotinamide adenine dinucleotide phosphate hydrogen; P_i, inorganic phosphate; PP_i, pyrophosphate; PRPP, phosphoribosyl pyrophosphate; PTS, phosphotransferase system; sAMP, adenylosuccinate; SAICAR, N-succinylo-5-aminoimidazole-4-carboxamide ribonucleotide; THF, tetrahydrofolate; UMP, uridine monophosphate; XMP, xanthosine monophosphate.

[b]Metabolic knockout growth experiments were conducted either in an *in vivo* mouse infection model (in which case, the mouse urine, bladder, and kidneys may be collected for analysis) or *in vitro* bacterial cultures in human urine. The wild-type and knockout were coinfected in mice, and strains were grown separately in *in vitro* cultures.

[c]Transcriptomics analyses may be performed on human urine obtained directly from UTI patients, human urine from *in vitro* human urine cultures of UTI isolates, or mouse urine from *in vivo* UTI mouse models infected with UTI isolates. Analyses may be performed with microarrays, quantitative polymerase chain reaction (qPCR), RNA-sequencing, or tandem mass spectrometry (proteomics).

[d]I, wild-type and knockout were independently infected rather than coinfected in mice; +, same as wild-type growth; ++, enhanced growth; –, defective growth (significance based on P value <0.05). Results from different studies are on separate lines, and multiple results from the same study are separated by a comma.

[e]M, *in vitro* cultures in laboratory media used as the comparative control; T, one of the top nonribosomal genes demonstrating the highest or lowest gene expression, without comparison to a control group; U, *in vitro* cultures in human urine used as the comparative control; ↑, upregulated gene expression ($\log_2$ fold change >2 in the majority of UPEC isolates); ↓, downregulated gene expression ($\log_2$ fold change < –2 in the majority of UPEC isolates). Results from different studies are on separate lines, and multiple results from the same study are separated by a comma.

[f]Although, *fumA* is involved in oxidative TCA, *fumA* is not active in iron-deficient environments like urine. Thus, the dispensability of *fumA* is attributed to its lack of activity in urinary conditions rather than to the dispensability of oxidative TCA.

Table 2. Metabolic genes involved in amino acid catabolism and anabolism, and their contribution to UTI

Metabolic gene description[a]			Results from knockout growth experiments						Results from transcriptomics analyses			
Gene	Metabolic function	Reaction catalysed	Mouse bladder	Mouse kidney	Mouse urine	Human urine	*E. coli* strain	Refs	*In vivo* Human urine	*In vitro* Human urine	*In vivo* Mouse urine	Refs
Small peptide transport												
oppA	ABC transporter substrate-binding protein for oligopeptide transport	N/A[d]	–	–			CFT073	[25]	↑T ↑M	↑M		[17,23,25]
dppA	ABC transporter substrate-binding protein for dipeptide transport	N/A	–	+			CFT073	[25]	↑T	↑M ↑M	↑M	[17,18,25]
dppB	ABC transporter permease for dipeptide transport	N/A							↑M	↑M		[22]
dppC	ABC transporter permease for dipeptide transport	N/A							↑U, ↑M ↑M			[22,23]
dppD	ABC transporter ATP-binding subunit for dipeptide transport	N/A							↑M ↑M	↑M		[22,23]
dppF	ABC transporter ATP-binding subunit for dipeptide transport	N/A							↑U, ↑M			[22]
ygdR	Putative tripeptide transporter permease for dipeptide and tripeptide transport	N/A							↑T, ↑U			[17]
Glutamate and glutamine metabolism												
gltI	ABC transporter periplasmic binding protein in L-glutamate and L-aspartate transport	N/A							↓U	↑M	↑M ↓U	[18,24]
gdhA	Glutamate dehydrogenase in L-glutamate biosynthesis	α-ketoglutarate + NADPH + NH_4^+ ↔ L-glutamate + H_2O + $NADP^+$							↓U ↑M			[17,23]
gltB	Glutamate synthase subunit in L-glutamate biosynthesis	L-glutamine + α-ketoglutarate + NADPH + H^+ ➔ 2 L-glutamate + $NADP^+$ L-glutamate + $NADP^+$ + H2O ↔ NH_4^+ + α-ketoglutarate + NADPH + H^+							↑M			[23]
gadA	Glutamate decarboxylase α in glutamate decarboxylase-dependent acid resistance system	L-glutamate + H^+ ➔ GABA + CO_2									↓M	[18]
gadB	Glutamate decarboxylase β in glutamate decarboxylase-dependent acid resistance system	Same as *gadA*							↓M		↓M	[18,23]
ybaS	Glutaminase in L-glutamine catabolism	L-glutamine + H_2O ➔ L-glutamate + NH_4^+									↓M	[18]
glnP	ABC transporter permease for L-glutamine transport	N/A									↑M	[18]

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Table 2. (continued)

Metabolic gene description[a]			Results from knockout growth experiments						Results from transcriptomics analyses			
Gene	Metabolic function	Reaction catalysed	Mouse bladder	Mouse kidney	Mouse urine	Human urine	*E. coli* strain	Refs	*In vivo* Human urine	*In vitro* Human urine	*In vivo* Mouse urine	Refs
glnQ	ABC transporter ATP-binding subunit in L-glutamine transport	N/A									↑M	[18]
glnA	Glutamine synthetase in L-glutamine biosynthesis and nitrogen assimilation	L-glutamate + NH_4^+ + ATP ➔ L-glutamine + ADP + P_i + H^+							↑T, ↑U ↑M		↑M	[17,18,23]
Arginine and polyamine metabolism												
artJ	Periplasmic binding protein for L-arginine transport	N/A								↑M	↑M	[18]
argA	Amino acid acetyltransferase in L-arginine biosynthesis	Acetyl-CoA + L-glutamate ➔ CoA + N-acetyl-L-glutamate	–			–	UT189	[38]	↓U ↑M			[21,23]
argB1/ argB2	Acetylglutamate kinase in L-arginine biosynthesis	N-acetyl-L-glutamate + ATP ➔ N-acetyl-L-glutamyl 5-phosphate + ADP				–	CFT073	[39]	↓U	↑M		[18,21]
argC	N-acetyl-γ-glutamyl-phosphate reductase in L-arginine biosynthesis	N-acetyl-L-glutamyl 5-phosphate + NADPH + H^+ ➔ N-acetyl-L-glutamate 5-semialdehyde + $NADP^+$ + P_i	 + +	 – –	 + +	– –	CFT073 ABU83972 CP9	[39,40,93]	↓U ↑M	↑M	↑M	[18,21,23]
argD	N-acetylornithine aminotransferase aminotransferase in L-arginine and L-lysine biosynthesis	N-acetyl-L-ornithine + α-ketoglutarate ↔ N-acetyl-L-glutamate 5-semialdehyde + L-glutamate L-glutamate + N-succinyl-2-amino-6-ketopimelate ➔ α-ketoglutarate + N-succinyl-2,6-diaminopimelate							↓U ↑M			[21,23]
argE	Acetylornithine deacetylase in L-arginine biosynthesis	N^2-acetyl-L-ornithine + H_2O ➔ acetate + L-ornithine				–	*E. coli* 83972	[40]	↓U	↑M		[18,21]
argG	Argininosuccinate synthase in L-arginine biosynthesis	L-aspartate + L-citrulline + ATP ➔ argininosuccinate + AMP + PP_i + H^+	– +	– +		–	CFT073 UT189	[25,38]	↓U ↑M	↑M		[21,23,25]
argH	Argininosuccinate lyase in L-arginine biosynthesis	Argininosuccinate ➔ fumarate + L-arginine							↓U			[21]
argI	Ornithine transcarbamoylase subunit in L-arginine biosynthesis	Carbamoyl phosphate + ornithine ➔ citrulline + P_i	–			–	UT189	[38]	↑M			[23]
argBCH	Enzymes involved in L-arginine biosynthesis See above for *argB*, *argC*, *argH*					–	CFT073	[39]				
carA/B	Carbamoyl phosphate synthase in L-arginine (and *de novo* pyrimidine) biosynthesis	2 ATP + H_2O + hydrogencarbonate + L-glutamine ➔ 2 ADP + carbamoyl phosphate + $2H^+$ + L-glutamate + P_i	 –	 –	 +	–[b] –	CFT073 ABU83972	[39,40]	↑M			[23]

gabT	4-aminobutyrate aminotransferase in arginine, polyamine, and lysine metabolism	α-ketoglutarate + 4-aminobutanoate ↔ L-glutamate + succinate semialdehyde N-acetyl-L-ornithine + α-ketoglutarate ↔ N-acetyl-L-glutamate 5-semialdehyde + L-glutamate 5-aminopentanoate + α-ketoglutarate ↔ L-glutamate + glutarate semialdehyde							↓M, ↓U ↓M ↓U		↓M, ↓U	[21,23,24]
astB	N-succinylarginine dihydrolase in arginine catabolism	N^2-succinyl-L-arginine + 2 H^+ + 2 H_2O ➔ 2 NH_4^+ + N^2-succinyl-L-ornithine + CO_2							↓M		↓M	[24]
astC	Succinylornithine transaminase in arginine catabolism	N^2-succinyl-L-ornithine + α-ketoglutarate ↔ N^2-succinyl-L-glutamate 5-semialdehyde + L-glutamate							↓M		↓M	[24]
astD	Aldehyde dehydrogenase in arginine catabolism	N^2-succinyl-L-glutamate 5-semialdehyde + NAD^+ + H_2O ➔ N^2-succinylglutamate + NADH + 2 H^+							↓M		↓M	[24]
astE	Succinylglutamate desuccinylase in arginine catabolism	N^2-succinylglutamate + H_2O ➔ succinate + L-glutamate							↓M		↓M	[24]
ydgI	Putative arginine and ornithine transporter	N/A							↑U			[21]
potA	ABC transporter ATP-binding subunit for spermidine and putrescine transport	N/A							↑U, ↑M ↑M			[22,23]
potB	ABC transporter permease for spermidine and putrescine transport	N/A							↑U ↑U ↑U, ↑M		↑U	[21,22,24]
potG	ABC transporter ATP-binding subunit for putrescine transport	N/A							↑M	↑M		[22]
potI	ABC transporter permease for putrescine transport	N/A							↑M	↑M		[22]
speB	Agmatinase in putrescine biosynthesis	Agmatine + H_2O ➔ putrescine	++	+			CFT073	[25]		↑M		[25]
patA	Putrescine aminotransferase in putrescine and L-lysine degradation	putrescine + α-ketoglutarate ↔ 4-aminobutanal + L-glutamate cadaverine + α-ketoglutarate ↔ 5-aminopentanal + L-glutamate aliphatic diamine + α-ketoglutarate ↔ aliphatic aminoaldehyde + L-glutamate							↑U, ↑M ↓U	↑M		[21,22]
Serine metabolism												
sdaC	L-serine transporter	N/A							↑U		↑U	[21,24]

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Table 2. (continued)

Metabolic gene description[a]			Results from knockout growth experiments						Results from transcriptomics analyses			
Gene	Metabolic function	Reaction catalysed	Mouse bladder	Mouse kidney	Mouse urine	Human urine	*E. coli* strain	Refs	*In vivo* Human urine	*In vitro* Human urine	*In vivo* Mouse urine	Refs
									↑U			
sdaAB	L-serine dehydratase isozymes in L-serine catabolism	L-serine ➔ pyruvate + NH_4^+	–[c]	–[c]			CFT073	[41]				
dsdA	D-serine dehydratase in D-serine catabolism	D-serine ➔ pyruvate + NH_4^+	+ ++[c]	+ ++[c]		– –	CFT073	[26,27]				
dsdC	Transcriptional regulator of *dsdXA*	N/A	++[c]	++[c]			CFT073	[41]				
dsdA *sdaAB*	Serine dehydratases involved in L- and D-serine catabolism See above for *dsdA*, *sdaAB*		— [c]	– +[c]			CFT073	[26,41]				
dsdAC	Enzymes involved in D-serine catabolism See above for *dsdA*, *dsdC*		++[c]	++[c]			CFT073	[41]				
dsdX *cycA*	Enzymes involved in D-serine import		++	+			CFT073	[26]				
dsdXA *cycA*	Enzymes involved in D-serine catabolism and import See above for *dsdA*		+[c]	–[c]			CFT073	[41]				
serA	D-3-phosphoglycerate dehydrogenase in L-serine biosynthesis	3-phosphoglycerate + NAD^+ ➔ 3-phosphooxypyruvate + H^+ + NADH	+	+			CFT073	[25]	↓U	↑M ↑M		[18,21,25]
Branched-chain amino acid (BCAA) metabolism												
livF	ABC transporter ATP-binding subunit for BCAA and phenylalanine transport	N/A							↑M	↑M		[22]
livG	ABC transporter ATP-binding subunit for BCAA and phenylalanine transport	N/A							↑M ↑M	↑M ↑M		[18,22,23]
livH	ABC transporter permease for BCAA and phenylalanine transport	N/A							↑M ↑M ↓U	↑M		[21–23]
livJ	ABC transporter periplasmic binding protein for BCAA and phenylalanine transport	N/A								↑M		[18]
livK	ABC transporter periplasmic binding protein for BCAA and phenylalanine transport	N/A							↑M ↑M	↑M ↑M		[22,23,25]
livM	ABC transporter permease for BCAA and phenylalanine transport	N/A							↑M ↑M ↓U	↑M		[21–23]
leuA	2-isopropylmalate synthase in L-leucine biosynthesis	3-methyl-2-oxobutanoate + acetyl-CoA + H_2O ➔ 2-isopropylmalate + CoA + H^+							↓U ↓U		↓U	[21,24]

leuB	3-isopropylmalate dehydrogenase in L-leucine biosynthesis	3-isopropylmalate + NAD^+ ➔ 4-methyl-2-oxopentanoate + CO_2 + NADH							↓U ↓U	↑M	↓U	[18,21,24]
leuC	3-isopropylmalate dehydratase subunit in L-leucine biosynthesis	3-isopropylmalate ↔ 2-isopropylmalate							↓U ↓U	↑M	↓U	[18,21,24]
leuD	3-isopropylmalate dehydratase subunit in L-leucine biosynthesis	Same as *leuC*							↓U ↓U		↓U	[21,24]
leuL	Transcriptional regulator of *leu* operon	N/A								↑M		[18]
leuO	Transcriptional regulator of *leu* operon	N/A							↓U			[21]
ilvA	L-threonine dehydratase in L-isoleucine biosynthesis	L-threonine ➔ α-ketobutyrate + NH_4^+				–	ABU83972	[40]	↓U			[21]
ilvB	Acetolactate synthase 1 subunit in BCAA biosynthesis	2 pyruvate + H^+ ➔ α-acetolactate + CO_2 α-ketobutyrate + pyruvate + H^+ ➔ α-acetohydroxybutyrate + CO_2				–	CFT073	[39]	↓U			[21]
ilvN	Acetolactate synthase 2 subunit in BCAA biosynthesis	Same as *ilvB*							↓U ↓U		↓U	[21,24]
ilvM	Acetolactate synthase 2 subunit in BCAA biosynthesis	Same as *ilvB*							↓U ↓U		↓U	[21,24]
ilvI	Acetolactate synthase 3 subunit in BCAA biosynthesis	Same as *ilvB*							↑M			[23]
ilvC	Ketol-acid reductoisomerase in BCAA biosynthesis	α-acetolactate + H^+ + NADPH ➔ α,β-dihydroxy-isovalerate + $NADP^+$ α-acetohydroxybutyrate + H^+ + NADPH ➔ α,β-dihydroxy-β-methylvalerate + $NADP^+$	+	+	+	–	ABU83972	[40]	↓U ↑M	↑M	↓U	[18,23,24]
ilvD	Dihydroxy-acid dehydratase in BCAA biosynthesis	α,β-dihydroxy-isovalerate ➔ α-ketoisovalerate + H_2O α,β-dihydroxy-β-methylvalerate ➔ α-keto-β-methylvalerate+ H_2O				–	CFT073	[39]	↓U ↓U ↑M		↓U	[21,23,24]
ilvE	BCAA aminotransferase in BCAA biosynthesis	α-ketoisocaproate + L-glutamate ➔ α-ketoglutarate + L-leucine α-keto-β-methylvalerate + L-glutamate ➔ α-ketoglutarate + L-isoleucine α-ketoisovalerate + L-glutamate ➔ α-ketoglutarate + L-valine				–	CFT073	[39]	↓U ↓U	↑M	↓U ↑M	[18,21,24]
ilvH	Acetolactate synthase subunit in BCAA biosynthesis	Same as *ilvGM*							↓U	↑M		[18,21]
ilvY	*ilvC* transcriptional regulator	N/A				–	CFT073	[39]				

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Table 2. (continued)

Metabolic gene description[a]			Results from knockout growth experiments						Results from transcriptomics analyses			
Gene	Metabolic function	Reaction catalysed	Mouse bladder	Mouse kidney	Mouse urine	Human urine	*E. coli* strain	Refs	*In vivo* Human urine	*In vitro* Human urine	*In vivo* Mouse urine	Refs
ilvGMEDA	Enzymes involved in BCAA biosynthesis See above for *ilvG, ilvM, ilvE, ilvD, ilvA*					–	CFT073	[39]				
Lysine metabolism												
cadB	Lysine and cadaverine antiporter for lysine-dependent acid resistance system	N/A							↓U			[21]
lysP	Lysine transporter	N/A							↑U			[21]
lysC	Aspartate kinase III in L-lysine biosynthesis	L-aspartate + ATP ➔ L-aspartyl-4-phosphate + ADP							↓U ↑M			[21,23]
lysA	Diaminopimelate decarboxylase in L-lysine biosynthesis	Diaminopimelate + H^+ ➔ L-lysine + CO_2							↓U			[21]
gabD	Succinate-semialdehyde dehydrogenase in L-lysine catabolism	Glutarate semialdehyde + $NADP^+$ + H_2O ➔ glutarate + NADPH + 2 H^+							↓U ↑M		↓U	[23,24]
Methionine metabolism												
metI	ABC transporter membrane subunit for L-/D-methionine transport	N/A							↓U			[21]
metN	ABC transporter ATP-binding subunit for L-/D-methionine transport	N/A							↓U			[21]
metQ	ABC transporter anchored binding protein for L-/D-methionine transport	N/A							↓U			[21]
metA	Homoserine O-succinyltransferase in L-methionine biosynthesis	L-homoserine + succinyl-CoA ➔ O-succinyl-L-homoserine + CoA				–	CFT073	[39]	↓U			[21]
metB	Cystathionine gamma-synthase in L-methionine biosynthesis	L-cysteine + O-succinyl-L-homoserine ➔ L,L-cystathionine + succinate + H^+				–	CFT073	[39]	↓U			[21]
metC	Cystathionine β-lyase in L-methionine biosynthesis	L-cystathionine + H_2O ➔ NH_4^+ + pyruvate + L-homocysteine							↓U			[21]
metE	5-methyltetrahydro-pteroyltriglutamate--homocysteine methyltransferase in L-methionine biosynthesis	5-MTHF + L-homocysteine ➔ L-methionine + tetrahydropteroyltri-L-glutamate	+	+	+	–	ABU83972	[40]	↑M ↑M ↓U		↑M	[21,23,24]
metL	Bifunctional aspartokinase/ homoserine dehydrogenase 2 in L-methionine biosynthesis	L-aspartate 4-semialdehyde + NADPH + H^+ ➔ L-homoserine + $NADP^+$							↓U			[21]
metR	*met* operon transcriptional regulator	N/A							↓U			[21]
metJBL	Enzymes involved in L-methionine biosynthesis					–	CFT073	[39]				

	See above for *metB*, *metL*											
Proline metabolism												
proV	ABC transporter ATP-binding subunit for L-proline transport	N/A							↑U, ↑M ↑M	↑M	↑M ↑M	[18,22,24]
proW	ABC transporter permease for L-proline transport	N/A							↑U, ↑M ↑U ↑M	↑M	↑M	[21,22,24]
proX	ABC transporter periplasmic binding protein for L-proline transport	N/A							↑U, ↑M ↑U ↑M	↑M	↑M	[21,22,24]
putA	Proline dehydrogenase in proline catabolism	L-glutamate-5-semialdehyde + NAD^+ + H_2O → L-glutamate + NADH + 2 H^+							↓U			[21]
Other amino acid metabolic pathways and transporters												
glyA	Serine hydroxymethyltransferase in glycine biosynthesis	L-serine + THF ↔ glycine + 5,10-methylene-THF + H_2O							↓U ↑M	↑M		[21,23,25]
gcvP	Glycine decarboxylase in glycine catabolism	Glycine + [protein]-L-lysine + H^+ ↔ [protein]-aminomethyldihydrolipoyl-L-lysine + CO_2 glycine + THF + NAD^+ ➔ 5,10-methylene-THF + NH_4^+ + CO_2 + NADH							↓U			[21]
avtA	Valine-pyruvate aminotransferase in L-alanine biosynthesis	Pyruvate + L-valine ↔ L-alanine + 3-methyl-2-oxobutanoate							↑M			[23]
hisJ	ABC transporter periplasmic binding protein precursor for L-histidine transport	N/A								↑M ↑M		[18,25]
ansP	L-asparagine transporter	N/A							↑U, ↑M			[22]
asnB	Asparagine synthetase B in L-asparagine biosynthesis and L-glutamine catabolism	L-glutamine + L-aspartate + ATP + H_2O ➔ L-glutamate + L-asparagine + AMP + PP_i + H^+ L-aspartate + NH_4^+ + ATP ➔ L-asparagine + AMP + PP_i + H^+ L-glutamine + H_2O ➔ L-glutamate + NH_3 + H^+								↑M		[18]
aspC	Aspartate aminotransferase in multiple amino acid pathways	L-aspartate + α-ketoglutarate ↔ oxaloacetate + L-glutamate							↑M			[23]

[a]See footnotes of the headers from Table 1.
[b]Although *carAB* is in both arginine and *de novo* pyrimidine biosynthetic pathways, *carAB*'s necessity is attributed to its involvement in arginine rather than pyrimidine biosynthesis, since the addition of arginine restores the *carA* mutant's growth back to normal levels [39].
[c]Phenotype may be impacted by previously unrecognized secondary mutation in *rpoS* rather than attributed to the mutation in D-serine utilization genes.
[d]N/A indicates that the gene does not encode a enzyme that catalyzes a reaction. Genes with N/A are either transporters or transcriptional gene regulators.

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Table 3. Metabolic genes involved in nucleic acid biosynthesis, and their contribution to UTI

Metabolic gene description[a]			Results from knockout growth experiments						Results from transcriptomics analyses			
Gene	Metabolic function	Reaction catalysed	Mouse bladder	Mouse kidney	Mouse urine	Human urine	*E. coli* strain	Refs	*In vivo* Human urine	*In vitro* Human urine	*In vivo* Mouse urine	Refs
Salvage and *de novo* pathways of purine biosynthesis												
prs	Ribose-phosphate diphosphokinase in PRPP biosynthesis	D-ribose 5-phosphate + ATP ➔ 5-phospho-D-ribose 1-diphosphate + AMP + H^+							↑M			[23]
purF	Amidophosphoribosyl-transferase in *de novo* pathway of purine biosynthesis	PRPP + H_2O + L-glutamine ➔ phosphoribosylamine + PP_i + L-glutamate	–			+	UTI89	[28]				
purD	Glycinamide ribonucleotide synthetase in *de novo* pathway of purine biosynthesis	phosphoribosylamine + ATP + glycine ➔ GAR + P_i + ADP + H^+				+	CFT073	[39]				
purN	Phosphoribosylglycinamide formyltransferase in *de novo* pathway of purine biosynthesis	GAR +10-formyl-THF ➔ FGAR + THF + H^+	+I				UT189	[29]				
purT	Formate-dependent phosphoribosylglycinamide formyltransferase in *de novo* pathway of purine biosynthesis	GAR + formate + ATP ➔ FGAR + ADP + P_i + H^+	+I			+	UT189	[29]				
purM	Phosphoribosylformyl-glycinamidine cyclo-ligase in *de novo* pathway of purine biosynthesis	FGAM + ATP ➔ AIR + ADP + H^+ + P_i				+	CFT073	[39]				
purE	N5-CAIR ribonucleotide mutase in *de novo* pathway of purine biosynthesis	N5-CAIR + H^+ ➔ CAIR	+	+	+	+	CFT073	[39]				
purB	Adenylosuccinate lyase in *de novo* pathway of purine biosynthesis	SAICAR ➔ AICAR + fumarate							↑M			[23]
purH	AICAR transformylase and IMP cyclohydrolase in *de novo* pathway of purine biosynthesis	AICAR +10-formyl-THF ➔ FAICAR + THF FAICAR ➔ IMP + H_2O				+	CFT073	[39]				
xanP	Xanthine transporter	N/A[b]							↑U			[21]
gsk	Inosine/guanosine kinase in salvage pathway of purine biosynthesis	Inosine + ATP ➔ IMP + ADP + H^+ Guanosine + ATP ➔ GMP + ADP + H^+							↑U			[21]
gpt	Xanthine phosphoribosyltransferase in salvage pathway of purine biosynthesis	guanine + PRPP ➔ GMP + PP_i							↑U			[21]
add	Adenosine deaminase in salvage pathway of purine biosynthesis	(Deoxy)adenosine + H_2O + H^+ ➔ NH_4^+ + (deoxy)inosine							↓M			[23]
purA	Adenylosuccinate synthetase in *de novo* and salvage pathways of purine biosynthesis	GTP + IMP + L-aspartate ➔ GDP + sAMP + P_i + $2H^+$	–	–	–	– –	CFT073 ABU83972	[39,40]				

guaA	GMP synthase in *de novo* and salvage pathways of purine biosynthesis	XMP + ATP + H_2O + L-glutamine → GMP + AMP + PP_i + $2H^+$ + L-glutamate	– –	– +	– –	–	CFT073 CP9	[39,93]	↑U			[17]
guaB	Inosine 5′-monophosphate dehydrogenase in *de novo* and salvage pathways of purine biosynthesis	IMP + NAD^+ + H_2O → XMP + H^+ + NADH				–	CFT073	[39]	↑U ↑U		↑U	[21,24]
purNT	Enzymes involved in *de novo* pathway of purine biosynthesis See above for *purN*, *purT*		+I			–	UT189	[29]				
purE *gpt*	Enzymes involved in *de novo* and salvage pathways of purine biosynthesis See above for *purE*, *gpt*					+	CFT073	[39]				
purE *apt*	Enzymes involved in *de novo* and salvage pathways of purine biosynthesis See above for *purE*					+	CFT073	[39]				
purE *hpt*	Enzymes involved in *de novo* and salvage pathways of purine biosynthesis See above for *purE*					–	CFT073	[39]				
apt *hpt*	Enzymes involved in salvage pathway of purine biosynthesis		–	+	–	–	CFT073	[39]				
purE *apt* *hpt*	Enzymes involved in *de novo* and salvage pathways of purine biosynthesis See above for *purE*		–	–	–	–	CFT073	[39]				
Salvage and *de novo* pathways of pyrimidine biosynthesis												
pyrBI	Aspartate carbamoyltransferase in *de novo* pathway of pyrimidine biosynthesis	Carbamoyl phosphate + L-aspartate → N-carbamoyl-L-aspartate + P_i + H^+				+	CFT073	[39]				
pyrD	Dihydroorotate dehydrogenase in *de novo* pathway of pyrimidine biosynthesis	Dihydroorotate + quinone → orotate + quinol	+	+	+	+	CFT073	[74]	↑U			[21]
pyrF	Orotidine 5′-phosphate decarboxylase in *de novo* pathway of pyrimidine biosynthesis	Orotidine 5-phosphate + H^+ → UMP + CO_2	+	+	+	+	CFT073	[39]	↑U		↑U	[24]
pyrH	UMP kinase in *de novo* pathway of pyrimidine biosynthesis	ATP + UMP → ADP + UDP							↑U			[21]
dut	dUTP diphosphatase in *de novo* pathway of pyrimidine biosynthesis	dUTP + H_2O → dUMP + PP_i + H^+									↑M	[18]
upp	Uracil phosphoribosyltransferase in salvage pathway of pyrimidine biosynthesis	Uracil + PRPP → UMP + PP_i	+	+	+	+	CFT073	[39]	↑U		↑U	[24]
udk	Uridine kinase in salvage pathway of pyrimidine biosynthesis	Uridine + ATP → UMP + ADP + H^+ Cytidine + ATP → CMP + ADP + H^+				+	CFT073	[39]	↑U			[21]
cmk	Cytidylate kinase in salvage pathway of pyrimidine biosynthesis	ATP + CMP ↔ ADP + CDP ATP + dCMP → ADP + dCDP							↑U			[21]
rihB	Pyrimidine-specific ribonucleoside hydrolase in salvage pathway of pyrimidine biosynthesis	Pyrimidine nucleoside + H_2O + H^+ → pyrimidine base + D-ribofuranose							↓U			[21]

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Table 3. (continued)

Metabolic gene description[a]			Results from knockout growth experiments						Results from transcriptomics analyses			
Gene	Metabolic function	Reaction catalysed	Mouse bladder	Mouse kidney	Mouse urine	Human urine	*E. coli* strain	Refs	*In vivo* Human urine	*In vitro* Human urine	*In vivo* Mouse urine	Refs
ndk	Nucleoside diphosphate kinase in *de novo* and salvage pathways of pyrimidine biosynthesis	Pyrimidine (deoxy)nucleoside diphosphate + ATP ➔ pyrimidine (deoxy)nucleoside triphosphate + ADP							↑U			[21]
pyrF *udk*	Enzymes involved in *de novo* and salvage pathways of pyrimidine biosynthesis See above for *pyrF*, *udk*					+	CFT073	[39]				
pyrF *upp*	Enzymes involved in *de novo* and salvage pathways of pyrimidine biosynthesis See above for *pyrF*, *upp*		–	–	–	–	CFT073	[39]				
Nucleotide catabolism												
adeD	Adenine deaminase in adenine catabolism and superoxide radical degradation	Adenine + H^+ + H_2O ➔ NH_4^+ + hypoxanthine 2 H_2O_2 ➔ 2 H2O + O_2							↑U, ↑M			[22]
xdhA	Putative xanthine dehydrogenase molybdenum-binding subunit in purine catabolism	Xanthine + NAD^+ + H_2O ↔ urate + NADH + H^+ Hypoxanthine + NAD^+ + H_2O → xanthine + NADH + H^+							↓U			[21]
xdhC	Putative xanthine dehydrogenase iron-sulfur-binding subunit in purine catabolism	Same as *xdhA*							↓U			[21]

[a]See footnotes of the headers from Table 1.
[b]Copied from previous response: N/A indicates that the gene does not encode a enzyme that catalyzes a reaction. Genes with N/A are either transporters or transcriptional gene regulators.

Table 4. Metabolic genes involved other miscellaneous metabolic pathways, and their contribution to UTI

Metabolic gene description[a]			Results from knockout growth experiments						Results from transcriptomics analyses			
Gene	Metabolic function	Reaction catalysed	Mouse bladder	Mouse kidney	Mouse urine	Human urine	*E. coli* strain	Refs	*In vivo* Human urine	*In vitro* Human urine	*In vivo* Mouse urine	Refs
Nonglycolytic carbohydrate metabolism												
vpeBC	EII complex in PTS for carbohydrate transport	N/A[c]	+	–		–	AL511	[30]		↑M		[30]
L-arabinose metabolism												
araF	ABC transporter periplasmic binding protein for L-arabinose transport	N/A	+	+			CFT073	[25]		↑M		[25]
araG	ABC transporter ATP-binding subunit for L-arabinose transport	N/A							↓U			[21]
araH	ABC transporter permease for L-arabinose transport	N/A							↓U			[21]
araA	L-arabinose isomerase in L-arabinose catabolism	L-arabinopyranose ↔ L-ribulose							↑M ↓U	↑M		[21,22]
araB	Ribulokinase in L-arabinose catabolism	L-ribulose + ATP ➔ L-ribulose 5-phosphate + ADP + H^+							↑M ↓U	↑M		[21,22]
araD	Ribulose-5-phosphate 4-epimerase in L-arabinose catabolism	L-ribulose 5-phosphate ↔ D-xylulose 5-phosphate							↑M ↓U			[21,22]
N-acetylneuraminate and N-acetylmannosamine metabolism												
nanT	N-acetylneuraminate transporter	N/A								↑M		[18]
nanC	N-acetylneuraminate outer membrane channel	N/A							↓U			[21]
nanA	N-acetylneuraminate lyase in N-acetylneuraminate and N-acetylmannosamine catabolism	N-acetylneuraminate ➔ N-acetyl-D-mannosamine + pyruvate	+	+			CFT073	[25]	↓U	↑M ↑M	↑M	[18,21,25]
nanK	N-acetylmannosamine kinase in N-acetylneuraminate and N-acetylmannosamine catabolism	N-acetyl-D-mannosamine + ATP ➔ N-acetyl-D-mannosamine 6-phosphate + ADP + H^+							↓U			[21]
nanE	Putative N-acetylmannosamine-6-phosphate 2-epimerase in N-acetylneuraminate and N-acetylmannosamine catabolism	N-acetyl-D-mannosamine 6-phosphate ↔ N-acetyl-D-glucosamine 6-phosphate							↓U			[21]
nanM	N-acetylneuraminate mutarotase in N-acetylneuraminate and N-acetylmannosamine catabolism	N-acetyl-α-neuraminate ↔ N-acetyl-β-neuraminate							↓U			[21]
nanS	N-acetyl-9-O-acetylneuraminate esterase in N-acetylneuraminate and N-acetylmannosamine catabolism	N-acetyl-9-O-acetylneuraminate + H_2O ➔ N-acetylneuraminate + acetate + H^+							↓U			[21]

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Table 4. (continued)

Metabolic gene description[a]			Results from knockout growth experiments						Results from transcriptomics analyses			
Gene	Metabolic function	Reaction catalysed	Mouse bladder	Mouse kidney	Mouse urine	Human urine	*E. coli* strain	Refs	*In vivo* Human urine	*In vitro* Human urine	*In vivo* Mouse urine	Refs
D-fructose and D-fructuronate metabolism												
fruA	Fructose-specific IIB/IIC component in the PTS for D-fructose transport	N/A							↑U			[21]
fruB	Fructose-specific IIA/Hpr component in the PTS for D-fructose transport	N/A							↑M ↑U	↑M	↑M	[21,24,25]
fruK	1-phosphofructokinase in D-fructose catabolism	D-fructofuranose 1-phosphate + ATP ➔ D-fructofuranose 1,6-bisphosphate + ADP + H^+							↑M ↑U		↑M	[21,24]
uxuA	D-mannonate dehydratase in D-fructuronate catabolism	D-mannonate ➔ 2-dehydro-3-deoxy-D-gluconate + H_2O	+	+			CFT073	[25]	↑U	↑M ↑M	↑M	[18,21,25]
uxuB	D-mannonate oxidoreductase in D-fructuronate catabolism	D-mannonate + NAD^+ ↔ D-fructuronate + NADH + H^+									↑M	[18]
D-galactose, D-galacturonate, D-glucuronate, and lactose metabolism												
mglA	ABC transporter ATP-binding subunit for D-galactoside transport	N/A								↑M		[18]
exuT	Hexuronate transporter for D-galacturonate and D-glucuronate transport	N/A							↓U			[21]
lacZ	β-galactosidase in lactose catabolism	β-galactoside ➔ D-galactose + D-glucose	–	–			UTI189	[31]	↑M ↑M ↓U	↑M		[21,22,31]
galM	Galactose-1-epimerase in D-galactose catabolism	β-D-galactopyranose ↔ α-D-galactopyranose							↓U			[21]
galK	Galactokinase in D-galactose catabolism	D-galactose + ATP ➔ D-galactose 1-phosphate + ADP + H^+	–	–			UTI189	[31]	↓U	↑M		[18,21]
galT	Galactose-1-phosphate uridylyltransferase in D-galactose catabolism	UDP-D-glucose + D-galactose 1-phosphate ↔ UDP-D-galactose + D-glucopyranose 1-phosphate							↓U			[21]
galE	UDP-glucose 4-epimerase in D-galactose catabolism	UDP-D-glucose ↔ UDP-D-galactose							↓U	↑M		[18,21]
galS	*gal* operon transcriptional regulator	N/A							↓U			[21]
melA	α-galactosidase in melibiose catabolism	Melibiose + H_2O ➔ D-galactopyranose + D-glucopyranose Melibionate + H_2O ➔ α-D-galactopyranose + D-gluconate							↑M ↓U	↑M		[21,22]
dgoT	D-galactonate transporter	N/A							↓U			[21]
dgoD	D-galactonate dehydratase in	D-galactonate ➔ 2-dehydro-3-deoxy-							↑U, ↑M			[21,22]

	D-galctonate catabolism (DeLey-Doudoroff pathway)	D-galactonate + H_2O							↓U			
dgoK1/ dgoK2	2-dehydro-3-deoxygalactonokinase in D-galctonate catabolism (DeLey-Doudoroff pathway)	2-dehydro-3-deoxy-D-galactonate + ATP ➔ 2-dehydro-3-deoxy-6-phospho-D-galactonate + ADP + H^+				–	CFT073	[39]	↓U			[21]
uxaA	D-altronate dehydratase in D-galacturonate catabolism	D-altronate ➔ 2-dehydro-3-deoxy-D-gluconate + H_2O							↓U		↑M	[18,21]
uxaB	D-altronate oxidoreductase in D-galacturonate catabolism	D-altronate + NAD^+ ↔ D-tagaturonate + NADH + H^+							↓U		↑M	[18,21]
uxaC	D-glucoronate/D-galacturonate isomerase in D-glucuronide, D-glucuronate and D-galacturonate catabolism	Aldehydo-D-glucuronate ↔ D-fructuronate Aldehydo-D-galacturonate ↔ D-tagaturonate							↓U	↑M		[18,21]
Glycogen metabolism												
glgC	Glucose-1-phosphate adenylyltransferase in glycogen biosynthesis	D-glucopyranose 1-phosphate + ATP + H^+ ➔ ADP-D-glucose + PP_i							↓U			[21]
glgA	Glycogen synthase in glycogen biosynthesis	$(1,4\text{-D-glucosyl})_n$ + ADP-D-glucose ↔ ADP + $(1,4\text{-D-glucosyl})_{n+1}$							↓U			[21]
glgB	1,4-α-glucan branching enzyme in glycogen biosynthesis	(1→4)-D-glucan ➔ glycogen							↓U			[21]
glgX	Glycogen debranching enzyme in glycogen catabolism	α-limit dextrin + H_2O ➔ debranched α-limit dextrin + maltotetraose							↓U			[21]
D-maltose metabolism												
malG	ABC transporter permease subunit for D-maltose transport	N/A							↓U			[21]
malF	ABC transporter permease subunit for D-maltose transport	N/A							↓U			[21]
malK	ABC transporter ATP-binding subunit for D-maltose transport	N/A							↓U	↑M		[21,25]
lamB	Outer membrane channel for maltose transport	N/A							↓U			[21]
D-sorbitol metabolism												
gatB	Galactitol-specific IIB component in the PTS for galactitol and D-sorbitol transport	N/A							↓U			[21]
srlA	Sorbitol-specific IIC2 component in the PTS for D-sorbitol transport	N/A	–	+			UTI189	[31]	↑M			[31]
srlB	Sorbitol-specific IIA component in the PTS for D-sorbitol transport	N/A							↓U		↑M	[18,21]
srlE	Sorbitol-specific IIBC component in the PTS for D-sorbitol transport	N/A							↓U		↑M	[18,21]

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Table 4. (continued)

Metabolic gene description[a]			Results from knockout growth experiments						Results from transcriptomics analyses			
Gene	Metabolic function	Reaction catalysed	Mouse bladder	Mouse kidney	Mouse urine	Human urine	*E. coli* strain	Refs	*In vivo* Human urine	*In vitro* Human urine	*In vivo* Mouse urine	Refs
srlD	Sorbitol-6-phosphate 2-dehydrogenase in D-sorbitol degradation	D-sorbitol 6-phosphate + NAD$^+$ ↔ keto-D-fructose 6-phosphate + NADH + H$^+$							↓U ↑U, ↑M		↑M	[18,21,22]
srlM	*srl* operon transcriptional regulator	N/A							↓U			[21]
D-xylose metabolism												
xylF	ABC transporter periplasmic binding protein for D-xylose transport	N/A							↑M ↓U	↑M		[21,22]
xylG	ABC transporter ATP-binding subunit for D-xylose transport	N/A							↓U			[21]
xylH	ABC transporter permease for D-xylose transport	N/A							↑M ↓U	↑M		[21,22]
xylA	Xylose isomerase in D-xylose catabolism	D-xylose ↔ D-xylulose	+	+			CFT073	[25]	↓U	↑M ↑M		[21,22,25]
xylR	*xyl* operon transcriptional regulator	N/A							↓U			[21]
Other carbohydrate metabolic pathways												
gntU	Gluconate transporter	N/A							↑U ↑U		↑U	[21,24]
cmtB	Mannitol-specific IIA component in the PTS for D-mannitol transport	N/A							↓U			[21]
otsA	Trehalose-6-phosphate synthase in trehalose biosynthesis	UDP-D-glucose + D-glucopyranose 6-phosphate ➔ UDP + trehalose 6-phosphate + H$^+$							↓U			[21]
otsB	Trehalose-6-phosphate phosphatase in trehalose biosynthesis	Trehalose 6-phosphate + H_2O ➔ trehalose + phosphate							↓U			[21]
rbsB	ABC transporter periplasmic binding protein in D-ribose transport	N/A							↓U			[21]
rbsC	ABC transporter permease in D-ribose transport	N/A							↓U			[21]
rbsD	Ribose pyranase in ribose catabolism	D-ribopyranose ↔ D-ribofuranose								↑M		[18]
Ethanolamine metabolism												
eutB	Ethanolamine ammonia-lyase subunit in ethanolamine metabolism	Ethanolamine ➔ acetaldehyde + NH_4^+				–[b]	U1	[32]				
eutD	Phosphate acetyltransferase in ethanolamine metabolism	Acetyl-CoA + P_i ↔ acetyl phosphate + CoA										
eutE	Acetaldehyde dehydrogenase in ethanolamine metabolism	Acetaldehyde + NAD$^+$ + CoA ➔ Acetyl-CoA + NADH + H$^+$				–[b]	U1	[32]				
eutG	Alcohol dehydrogenase in	Acetaldehyde + H$^+$ + NADH ➔ ethanol +							↑U, ↑M			[21,22]

	ethanolamine metabolism	NAD^+							↓U			
eutP	Acetate kinase in ethanolamine metabolism	Acetate + ATP ↔ acetyl phosphate + ADP							↑U, ↑M			[22]
eutQ	Acetate kinase in ethanolamine metabolism	Same as *eutP*										
eutR	*eut* operon transcriptional regulator	N/A	– –	–			CFT073	[22,33]	↑U, ↑M			[22]
eut	Ethanolamine utilization operon encoding all *eut* genes	Various reactions	–		–	+	CFT073	[33]				
Acetate metabolism												
satP	Acetate/succinate transporter	N/A							↑U			[21]
actP	Acetate/glycolate transporter	N/A							↑U, ↑M			[22]
pta	Phosphate acetyltransferase in acetate production	Acetyl-CoA + P_i ↔ acetyl phosphate + CoA	+	–			CFT073	[42]	↑U ↑U		↑U	[17,24]
ackA	Acetate kinase in acetate production	Acetyl phosphate + ADP ↔ acetate + ATP	+	–			CFT073	[42]	↑T ↑U		↑U	[17,24]
pta *ackA*	Enzymes involved in acetate production See above for *pta*, *ackA*		–	–			CFT073	[42]				
acs	Acetyl-CoA synthase in acetate assimilation	Acetate + ATP + CoA ➔ acetyl-CoA + AMP + PP_i	+	+			CFT073	[42]	↓U ↓U ↓U		↓U	[17,21,24]
Nitrate and nitrite metabolism												
narK	Nitrate:nitrite antiporter for nitrate and nitrite transport	N/A								↑M		[18]
narG	Nitrate reductase A subunit α in nitrate reduction	Nitrate + menaquinol$_{[inner\ membrane]}$ + 2 H^+ ➔ nitrite + a menaquinone$_{[inner\ membrane]}$ + H_2O + 2 $H^+_{[periplasm]}$ Nitrate + ubiquinol$_{[inner\ membrane]}$ + 2 H^+ ➔ nitrite + ubiquinone$_{[inner\ membrane]}$ + 2 $H^+_{[periplasm]}$ + H_2O								↑M		[18]
narI	Nitrate reductase A subunit γ in nitrate reduction	Same as narG								↑M		[18]
narGHJI	Terminal nitrate reductase in nitrate reduction	Same as *narG*	–	–	–		CFT073	[34]				
fdnG	Formate dehydrogenase-N subunit in nitrate reduction	Formate$_{[periplasm]}$ + a menaquinone$_{[inner\ membrane]}$ + 2 H^+ ➔ $CO_{2[periplasm]}$ + a menaquinol$_{[inner\ membrane]}$ + $H^+_{[periplasm]}$							↑M			[23]
fdnH	Formate dehydrogenase-N subunit in nitrate reduction	Same as *fdnG*							↑M, ↑U	↑M	↑M, ↑U ↑M	[18,24]
fdnI	Formate dehydrogenase-N subunit in nitrate reduction	Same as *fdnG*							↑M, ↑U ↑M	↑M	↑M, ↑U ↑M	[18,23,24]

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Table 4. (continued)

Metabolic gene description[a]			Results from knockout growth experiments						Results from transcriptomics analyses			
Gene	Metabolic function	Reaction catalysed	Mouse bladder	Mouse kidney	Mouse urine	Human urine	*E. coli* strain	Refs	*In vivo* Human urine	*In vitro* Human urine	*In vivo* Mouse urine	Refs
Lipid metabolism												
plsX	Putative phosphate acyltransferase in phospholipid metabolism	Acyl-ACP + phosphate ↔ acyl phosphate + ACP							↑U			[21]
cdh	CDP-diacylglycerol diphosphatase in phospholipid metabolism	CDP-diacylglycerol + H_2O ➔ phosphatidate + CMP + 2 H^+							↑U			[21]
ugpA	ABC transporter permease for glycerol 3-phosphate transport	N/A							↑U, ↑M ↓U	↑U		[21,22]
ugpE	ABC transporter permease for glycerol 3-phosphate transport	N/A							↑U, ↑M	↑U		[22]
gldA	Glycerol dehydrogenase in glycerol catabolism	Glycerol + NAD^+ ↔ dihydroxyacetone + NADH + H^+							↓U			[21]
glpQ	Glycerophosphoryl diester phosphodiesterase in glycerol and glycerophosphodiester catabolism	Glycerophosphodiester + H_2O ➔ alcohol + glycerol 3-phosphate + H^+							↓U			[21]
glpK	Glycerol kinase in glycerol and glycerophosphodiester catabolism	Glycerol + ATP ➔ glycerol 3-phosphate + ADP + H^+							↓U			[21]
glpA	Anaerobic glycerol-3-phosphate dehydrogenase subunit A in glycerol and glycerophosphodiester catabolism	Glycerol 3-phosphate + $\text{menaquinone}_{[\text{inner membrane}]}$ ➔ glycerone phosphate + $\text{menaquinol}_{[\text{inner membrane}]}$							↓U	↑M		[21,25]
glpB	Anaerobic glycerol-3-phosphate dehydrogenase subunit B in glycerol and glycerophosphodiester catabolism	Same as *glpA*							↓U			[21]
glpD	Aerobic glycerol 3-phosphate dehydrogenase in glycerol and glycerophosphodiester catabolism	Glycerol 3-phosphate + $\text{ubiquinone}_{[\text{inner membrane}]}$ ➔ glycerone phosphate + $\text{ubiquinol}_{[\text{inner membrane}]}$							↓U			[21]
fabH	3-oxoacyl-ACP synthase 3 in fatty acid biosynthesis	Acetyl-CoA + malonyl-ACP + H^+ ➔ acetoacetyl-ACP + CO_2 + CoA							↑U			[21]
yciA	Acyl-CoA thioesterase in unsaturated fatty acid biosynthesis	Acyl-CoA + H_2O ➔ carboxylate + CoA + H^+							↑U			[21]
fadA	3-ketoacyl-CoA thiolase in fatty acid β-oxidation	2,3,4-saturated 3-oxoacyl-CoA + CoA ➔2,3,4-saturated fatty acyl CoA + acetyl-CoA							↓M			[23]
fadB	Fatty acid oxidation complex subunit in fatty acid β-oxidation	Various reactions							↓M			[23]
fadE	Acyl-CoA dehydrogenase in fatty acid β-oxidation	2,3,4-saturated fatty acyl-CoA + oxidized flavoprotein + H^+ ➔ 2-enoyl-CoA + reduced flavoprotein							↓M			[23]
Other metabolic pathways												

fdhF	Formate dehydrogenase in formic acid degradation	Formate + $NAD^+ \rightarrow CO_2$ + NADH	–	–			CFT073	[22]	↑U			[21]
folA	Dihydrofolate reductase in THF biosynthesis	7,8-dihydrofolate + NADPH + $H^+ \rightarrow$ THF + $NADP^+$							↑U			[21]
falE	GTP cyclohydrolase 1 in THF biosynthesis	GTP + $H_2O \rightarrow$ formate + 7,8-dihydroneopterin 3'-triphosphate + H^+							↑M			[23]
metF	5,10-methylenetetrahydrofolate reductase in folate transformations	5,10-methylene-THF + NADH + $H^+ \rightarrow$ 5-methyl-THF+ NAD^+							↑M ↓U		↑M	[21,24]
thiI	tRNA uridine 4-sulfurtransferase in thiamine diphosphate biosynthesis	[Protein]-S-sulfanyl-L-cysteine + carboxy-adenylated-[protein] + 2 reduced ferredoxin $\rightarrow$ thiocarboxylated-[protein] + [protein]-L-cysteine + AMP + 2 oxidized ferredoxin							↑U			[21]
thiL	Thiamine monophosphate kinase in thiamine diphosphate biosynthesis	Thiamine phosphate + ATP $\rightarrow$ thiamine diphosphate + ADP							↑U			[21]
phnD	ABC transporter periplasmic binding protein for phosphonate transport	N/A							↑U, ↑M			[22]
phnA	Phosphonoacetate hydrolase in phosphonate utilization	Phosphonoacetate + $H_2O \leftrightarrow$ acetate + P_i							↑U, ↑M			[22]
phnR	*phn* operon transcriptional regulator	N/A	+	+			CFT073	[22]				
tauA	ABC transporter periplasmic binding protein for taurine transport	N/A	+	+			CFT073	[22]	↑U, ↑M			[22]
tauC	ABC transporter permease for taurine transport	N/A							↑U, ↑M			[22]

[a]See footnotes of the headers from Table 1.
[b]Artificial urine medium spiked with 10 mM ethanolamine.
[c]Copied from previous response: N/A indicates that the gene does not encode a enzyme that catalyzes a reaction. Genes with N/A are either transporters or transcriptional gene regulators.

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