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Letter to the Editor

Global effect of the AcrAB–TolC multidrug efflux pump of *Escherichia coli* in cell metabolism revealed by untargeted metabolomics

Sir,

AcrAB–TolC is the main multidrug efflux pump in *Escherichia coli* and other Enterobacteriaceae [1]. Besides removal of most classes of antibiotics, this pump also affects virulence, gene regulation, metabolism and other functions [1–4]. Uncovering its metabolic role is essential to understanding the role of this pump in adaptation to antibiotics and other stresses. However, our current knowledge is limited to a few metabolic pathways [2,4]. Here we employed metabolomics to determine whether the AcrAB–TolC pump of *E. coli* impacts metabolism at a global scale. The findings show, for the first time, that deletion or overproduction of this pump is associated with changes in a large number of central metabolism intermediates.

Using untargeted metabolomics, the intracellular and extracellular metabolic profiles of four *E. coli* strains grown aerobically at 37 °C to mid-exponential phase in EZ Rich Defined Medium with 0.2% glucose were compared. These strains were the parental strain BW25113 and its mutant derivatives: Δ*acrB*, which is inactivated for the AcrAB–TolC pump [1,4]; Δ*tolC*, which is inactivated for AcrAB–TolC and all other TolC-dependent efflux pumps [1]; and Δ*acrR*, which overexpresses AcrAB–TolC [1,4]. Full details of the strains, growth conditions and metabolomics experiments are provided in Supplementary File 1.

Compared with the parental strain, all efflux mutants showed significant ($P < 0.05$) changes in 58 to 105 metabolites (Fig. 1; Supplementary Table S1). Overall, 47 known metabolites were identified whose levels were significantly altered in at least one of the mutants. These metabolites were mostly involved in amino acid metabolism, the tricarboxylic acid cycle (TCA), fatty acid and other organic acids metabolism, and carbohydrate metabolism. The fold change in these metabolites compared with the parental strain ranged from a 2.5-fold increase in the intracellular levels of malic acid to a 5-fold decrease (0.2-fold) in the intracellular levels of glutamine, both in the Δ*acrB* mutant (Fig. 1).

Comparing the Δ*acrB* mutant with the parental strain, the most significantly increased known intracellular metabolites were: (i) the amino acids asparagine, aspartic acid, cysteine, glutamic acid, lysine and proline; (ii) the TCA intermediates malic and fumaric acids; and (iii) other compounds such as adenine, pelargonic acid, oxalic acid and xylitol. The most decreased known intracellular metabolites were: (i) the amino acids/intermediates 2-ketoisocaproic acid, aminomalonate, glutamine, *N*-acetylaspartic acid and sarcosine; (ii) the TCA intermediates citric and succinic acids; and (iii) other metabolites such as pyrophosphate, caprylic, glycolic and adipic acids, hydroxycarbamate, cellobiose and sucrose. In addition, several metabolites were found whose levels were increased (oxoproline, fumaric acid, malic acid and glycerol-3-galactoside) or decreased (2-ketoisovaleric, *N*-acetylaspartic, citric, succinic and pelargonic acids) in the supernatants of the Δ*acrB* strain compared with the parental strain (Fig. 1). A role of AcrAB–TolC in the metabolism of cysteine, glutamic acid and adenine is in agreement with previous findings [2,4]. Moreover, the large number of altered metabolites found in the Δ*acrB* strain is in agreement and might help explain the extensive changes in the expression of metabolic genes previously identified in this strain using microarrays [4]. Overall, these findings highlight the major role of the AcrAB–TolC pump in regulating the intra/extracellular levels of central metabolism intermediates, which may be related to the pleiotropic effects of this pump.

The results for the Δ*tolC* strain were similar to those found for the Δ*acrB* mutant (Fig. 1). However, several important differences were found. For example, intracellular and extracellular accumulation of 2-keitoisovaleric and citric acids was observed in the Δ*tolC* strain, in contrast to an intracellular and extracellular decrease of these metabolites in the Δ*acrB* strain. Conversely, the intracellular and extracellular levels of aspartic acid were decreased in the Δ*tolC* strain but were increased, mostly intracellularly, in the Δ*acrB* strain. AcrAB–TolC is considered to be the main TolC-dependent multidrug efflux pump in *E. coli* [1]. However, these findings suggest that other TolC-dependent pumps also play an important role in cell metabolism and highlight the complex interplay between all of these efflux pumps in *E. coli*.

The most significant difference between the Δ*acrR* and Δ*acrB* mutants was the intracellular decrease and extracellular increase of lysine in the Δ*acrR* strain compared with the intracellular increase and extracellular decrease found for this amino acid in the Δ*acrB* strain. These findings suggest that AcrAB–TolC might be directly involved in the efflux of lysine or its intermediates. Despite these and other differences, the overall intracellular and extracellular metabolic profiles of the Δ*acrR* and Δ*acrB* strains were similar. Such similarity might help explain previous findings that both mutants behave in a similar manner despite having opposite effects on AcrAB–TolC expression. For example, increased expression of flagellum biosynthesis genes and motility in *E. coli* was first found in the Δ*acrB* strain [4] and later in the Δ*acrR* strain [5].

In conclusion, this study demonstrates for the first time that the AcrAB–TolC multidrug efflux pump of *E. coli* plays a global role in central metabolism.

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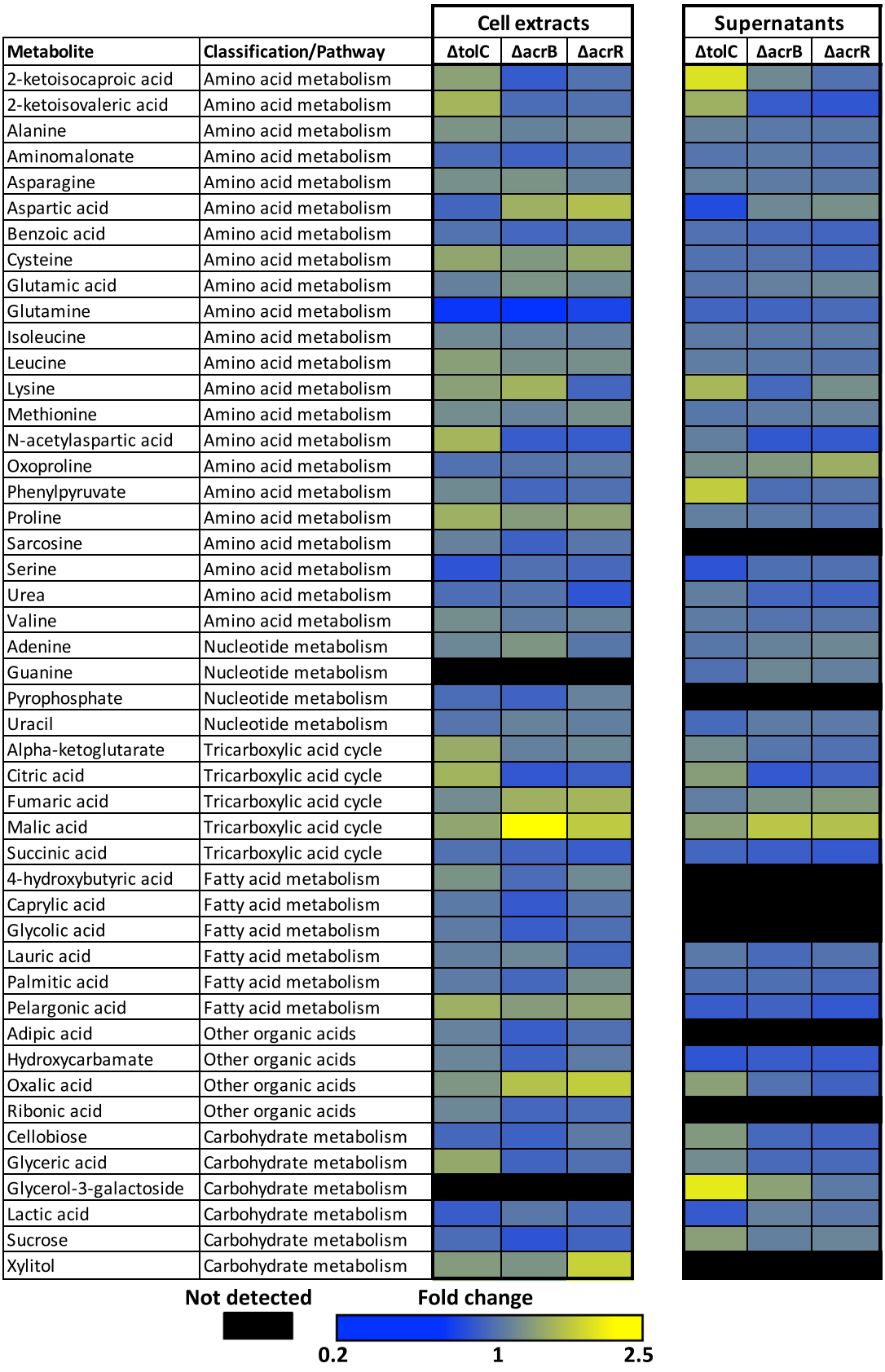

Fig. 1. Known metabolites with statistically significant ($P < 0.05$) altered levels in at least one efflux mutant strain compared with the parental strain. Metabolites with increased levels in the efflux mutant strain compared with the parental strain are highlighted according to the blue-yellow gradient scale at the bottom of the figure: more intense yellow colour indicates greater relative accumulation of the metabolite in the mutant strain compared with the parental, whereas more intense blue colour indicates a greater decrease in the relative abundance of the metabolite in the mutant strain compared with the parental strain. Black indicates that the metabolite was not detected in cell extracts or supernatants of that mutant.

Competing interests

None declared.

Ethical approval

Not required.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijantimicag.2019.05.015.

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