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Aspartate in the intestine: dual service as anaerobic electron acceptor and nitrogen source

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Summary

Fumarate was previously known to serve as an anaerobic electron acceptor by *E. coli* when colonizing the mammalian intestine, but the source of that fumarate was elusive. In this issue, Unden and coworkers demonstrate that L-aspartic acid is the source of fumarate that drives anaerobic respiration by colonized *E. coli* (Schubert et al., 2021). Moreover, Schubert et al., establish that *E. coli* is able to grow anaerobically by using aspartate as a sole source of nitrogen. These groundbreaking findings indicate that a single amino acid – aspartate – supports anaerobic respiration and acquisition of nitrogen by *E. coli* in the intestine.

To colonize the intestine, *E. coli* must acquire the nutrients that allow it to grow faster than the turnover rate of the luminal contents (Conway and Cohen, 2015). For example, mono- and disaccharides support the growth of *E. coli* in the intestine (Chang et al., 2004). Furthermore, the nutrients that support colonization are thought to underly niche occupation. Cohen's Restaurant Hypothesis of intestinal colonization states that degradation of mucosal polysaccharides by anaerobes facilitates sugar-specific niche occupation by *E. coli* (Conway and Cohen, 2015). Proof of this concept comes from experiments showing that intestinal growth of *Salmonella enterica* serovar Typhimurium and *Clostridioides difficile* depends upon monosaccharides that are liberated from mucus by *Bacteroides thetaiotaomicron* (Ng et al., 2013). It is important to note that most research in the field has focused on the bacterial metabolism of sugars as carbon and energy sources. See below for a discussion of electron donors in the intestine. Regarding mechanisms of nitrogen assimilation, little attention has been paid to it.

One reason that the paper by Schubert, *et al.*, is so interesting is, compared to sugars, far less is known about the consumption of amino acids by intestinal microbes. It was shown recently that uptake of aspartate and malate supports hydrogen-driven respiration of fumarate by colonized *S. typhimurium* (Nguyen et al., 2020). Also, aspartate is used by enterohemorrhagic *E. coli* to grow in intestinal contents (Bertin et al., 2018). In both cases, consumption of aspartate depends upon the aspartate: succinate antiport system (DcuA), just as shown by Schubert *et al.*, for *E. coli* to use aspartate as a source of fumarate and ammonia. In addition to aspartate, *S. typhimurium* uses fructose-asparagine as a primary nutrient during intestinal growth. Intermediates include aspartate and glucose-6-phosphate,

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suggesting that the fructose enters glycolysis and the aspartate is utilized like it is by *E. coli*. Aside from these related findings, next to nothing is known about the use of amino acids by colonized bacteria.

A very exciting finding by Schubert *et al.*, is the use of aspartate as a sole nitrogen source under anaerobic conditions. This result strongly suggests that ammonia derived from aspartate is assimilated when it is released by aspartase enzyme. What other sources of nitrogen for colonized bacteria have been studied? Very few. Only the investigation of ethanolamine has received significant attention. Ethanolamine utilization supports colonization by *S. typhimurim*, but whether it serves as a nitrogen source was not determined (Thiennimitr *et al.*, 2011). In EHEC, ethanolamine serves as a nitrogen source (Bertin *et al.*, 2011) and in uropathogenic *E. coli* it appears also to serve as a carbon source (Dadswell *et al.*, 2019). It is remarkable that more attention has not been paid to nitrogen assimilation by gut bacteria. Credit Schubert *et al.*, for publishing data most relevant to the question of what nitrogen sources support intestinal colonization.

The use of sugars as carbon and energy sources presumes that they serve as electron donors. Consumption of electron donors obviously requires electron acceptors. However, there is surprisingly little literature addressing the topic. Nevertheless, the use of oxygen as an electron acceptor in the intestine is well accepted, because *E. coli* requires cytochrome oxidase to be competitive (Jones *et al.*, 2007). Jones *et al.*, went on to postulate that if facultative anaerobes reduce oxygen availability, then they must also rely on anaerobic respiration to be competitive (Jones *et al.*, 2011). Whether individual bacteria respire oxygen and fumarate simultaneously remains an open question.

Unden and coworkers expand upon the idea that aerobic and anaerobic respiration both support *E. coli* colonization. They show that expression of the genes encoding fumarate reductase in the intestine is higher than would be expected based on laboratory cultivation. By way of explanation, they propose a model in which Lrp interacts with other regulators of anaerobic respiration to elevate production of the proteins required for fumarate respiration. They go on to note that fumarate respiration is driven by aspartate availability and that oxygen tension in the mouse gut should allow half-maximal expression of FNR-dependent fumarate and nitrate regulation. They therefore picture a situation in which microaerobic and fumarate respiration ‘coexist’ to provide the energy that *E. coli* needs to grow and persist in the intestine.

In conclusion, *E. coli* physiology appears to be poised to optimize mixed aerobic and anaerobic respiration of simple sugars and TCA cycle intermediates. This metabolic strategy maximizes competitiveness by allowing *E. coli* to make the most energy that can be afforded by conditions in the gut. It is no wonder that *E. coli* is a supreme intestinal colonizer!

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