Ethanolamine Influences Human Commensal Escherichia coli Growth, Gene Expression, and Competition with Enterohemorrhagic E. coli O157:H7

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ABSTRACT A core principle of bacterial pathogenesis is that pathogens preferentially utilize metabolites that commensal bacteria do not in order to sidestep nutritional competition. The metabolite ethanolamine (EA) is well recognized to play a central role in host adaptation for diverse pathogens. EA promotes growth and influences virulence during host infection. Although genes encoding EA utilization have been identified in diverse bacteria (nonpathogenic and pathogenic), a prevailing idea is that commensal bacteria do not utilize EA to enhance growth, and thus, EA is a noncompetitive metabolite for pathogens. Here, we show that EA augments growth of two human commensal strains of Escherichia coli. Significantly, these commensal strains grow more rapidly than, and even outcompete, the pathogen enterohemorrhagic E. coli O157:H7 specifically when EA is provided as the sole nitrogen source. Moreover, EA-dependent signaling is similarly conserved in the human commensal E. coli strain HS and influences expression of adhesins. These findings suggest a more extensive role for EA utilization in bacterial physiology and host-microbiota-pathogen interactions than previously appreciated.

IMPORTANCE The microbiota protects the host from invading pathogens by limiting access to nutrients. In turn, bacterial pathogens selectively exploit metabolites not readily used by the microbiota to establish infection. Ethanolamine has been linked to pathogenesis of diverse pathogens by serving as a noncompetitive metabolite that enhances pathogen growth as well as a signal that modulates virulence. Although ethanolamine is abundant in the gastrointestinal tract due to the turnover of bacterial and epithelial cells (EA is a breakdown product of the cell membrane lipid phosphatidylethanolamine) as well as through the diet (2). EA utilization plays a central role in host adaptation for a diverse range of pathogens, including opportunistic pathogens (3, 4). EA can serve as a carbon, nitrogen, and/or energy source to promote growth as well as a signal to influence virulence during host pathogenesis.

KEYWORDS ethanolamine, metabolism, microbiota, signaling
infection (5–11). Genes encoding EA utilization are carried in the ethanolamine utilization (eut) locus (12). In the Enterobacteriaceae, the eut locus encodes the transcription factor EutR. EutR senses EA and vitamin B₁₂ to directly activate eut transcription (13, 14). Moreover, in the foodborne pathogens enterohemorrhagic Escherichia coli O157:H7 (EHEC) and Salmonella enterica (EHEC and Typhimurium, EutR regulates expression of virulence traits (5, 13, 15, 16). Despite the continual replenishment of EA in the GI tract, it has been reported that commensal bacteria do not utilize EA (17), and thus, EA utilization is a trait associated with pathogenesis (3, 4).

The idea that EA is a noncompetitive metabolite for pathogens is largely perpetuated by data that showed that commensal E. coli isolated from ruminants did not consume EA in a modified bovine intestinal fluid (17). However, subsequent genome sequencing revealed that at least one of the E. coli strains used in the study contained several single nucleotide polymorphisms (SNPs) and an insertion element in the eut operon (18), which is expected to render this strain unable to utilize EA. In contrast, the eut operon of the human commensal E. coli HS strain contains an intact eut locus (19). HS was isolated from the stool of a healthy laboratory scientist and is used as a representative of nondomesticated E. coli in a number of human colonization studies (19–21). Therefore, to revisit EA utilization by human commensal E. coli, we assessed growth of HS when cultured in a minimal medium containing EA as the sole nitrogen or carbon source. Physiologically relevant concentrations of EA supported EutR-dependent growth of HS when cultured in a minimal medium containing EA as the sole nitrogen or carbon source. Physiologically relevant concentrations of EA supported EutR-dependent growth of HS when cultured in a minimal medium containing EA as the sole nitrogen or carbon source. Physiologically relevant concentrations of EA supported EutR-dependent growth of HS when cultured in a minimal medium containing EA as the sole nitrogen or carbon source. Physiologically relevant concentrations of EA supported EutR-dependent growth of HS when cultured in a minimal medium containing EA as the sole nitrogen or carbon source. Physiologically relevant concentrations of EA supported EutR-dependent growth of HS when cultured in a minimal medium containing EA as the sole nitrogen or carbon source.

The Gi tract contains several nitrogen sources that might diminish the potential importance of EA utilization in HS. To test this, we measured growth of HS in minimal medium containing NH₄ only or NH₄ and EA. When EA was added as a supplement to the medium, HS grew to a higher cell density than it did in medium containing only NH₄ (Fig. 1D). In support of these data, we also measured a significant increase in eut gene expression from HS grown in medium supplemented with EA compared to medium without EA supplementation (minimal medium containing NH₄ or Dulbecco’s modified Eagle’s medium) (Fig. 1E; see also Fig. S1 in the supplemental material). To confirm that EA utilization by a human E. coli isolate was not unique to the HS isolate, we next examined EA utilization in E. coli Nissle, which was isolated from the stool of a German soldier during World War I (22, 23). Consistent with the HS data, Nissle grew and responded to EA (Fig. S2A to D). Altogether, these data indicate that human commensal E. coli strains have maintained the ability to sense and utilize EA as a metabolite and that EA enhances growth in the presence of alternative nitrogen sources (as would be found in the gut).

We previously reported that EA influences expression of genes carried outside the eut locus in EHEC and Salmonella, including expression of fimbriae (5, 13, 15, 16). HS and EHEC share a conserved set of fimbrial loci; therefore, we next measured expression of one gene in each of the conserved loci (expression of eight genes was measured) in HS grown in minimal medium with NH₄ only or NH₄ and EA. We measured an ∼2- and 3-fold change in expression of genes carried in the yad and ybg loci, respectively (Fig. 1F). Interestingly, EA supplementation resulted in reduced levels of fimbrial gene expression in HS, which is the opposite of the impact of EA on EHEC fimbrial gene expression. These differences in expression may be reflective of the different colonization niches of these strains (lumen/mucus [HS] versus epithelial attachment [EHEC]). Regardless, these findings provide proof-of-principle data that similarly to EA-dependent growth, EA-dependent signaling is conserved in human commensal E. coli and not restricted to pathogens.

Scavenging nutrients is paramount for success in colonizing the host intestinal niche (24, 25). Commensal E. coli and EHEC compete for similar resources (24), and EA has been proposed to provide a selective growth advantage to EHEC over commensal E. coli (17). Therefore, we next compared growth of HS and EHEC in EA-minimal medium (containing glucose as the carbon source). Surprisingly, HS grew more rapidly than
EHEC when EA was provided as the sole nitrogen source (Fig. 2A), with a doubling time of 1.6 h compared to 4.3 h, respectively (of note, the doubling time of Nissle was 1.3 h [Fig. S2A]). Consistent with these data, during competition HS was recovered at nearly 10-fold-higher levels than EHEC (Fig. 2B). eut expression and/or enzymatic activity may be subject to carbon catabolite repression (26, 27); therefore, it is possible that effectiveness of carbon catabolite repression between HS and EHEC caused the differences in growth rates. To test this idea, we repeated the growth and competition experiments in EA-minimal medium containing glycerol as the sole carbon source. During exponential growth, growth rates of HS and EHEC were similar to growth rates in medium containing glucose, with doubling times of 1.4 h and 4.2 h, respectively (Fig. 2C). Of note, we observed a slightly shorter lag phase for EHEC grown in EA-minimal medium containing glycerol compared to glucose. Even so, consistent with the previous assay, HS was recovered in higher numbers than EHEC during competition (>2-fold) (Fig. 2D). Interestingly, this growth advantage was specific for EA utilization as no differences in bacterial growth or recovery were measured when HS and EHEC were cultured in minimal medium containing NH₄ as the sole nitrogen source (Fig. 2E and F and Fig. S3A and B).

Although genes encoding EA utilization are carried by phylogenetically diverse bacteria (27), EA utilization has been suggested to be a potential virulence determinant and/or has been specifically linked to pathogenesis (i.e., references 4, 7, and 28 to 31). Our findings reveal that commensal GI bacteria rely on EA to enhance growth, and thus,
EA utilization and signaling are more complex than previously appreciated. This work suggests that further investigation on the impact of EA utilization on host-microbiota-pathogen interaction is warranted.

**SUPPLEMENTAL MATERIAL**

Supplemental material for this article may be found at https://doi.org/10.1128/mBio.01429-18.

**TEXT S1**, PDF file, 0.04 MB.
**FIG S1**, TIF file, 0.1 MB.
**FIG S2**, TIF file, 0.3 MB.
**FIG S3**, TIF file, 0.1 MB.
**TABLE S1**, PDF file, 0.02 MB.

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