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OBSERVATION
Host-Microbe Biology

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, the prevailing idea is that commensal bacteria do not utilize EA, and thus, EA utilization has been particularly associated with pathogenesis. Here, we provide evidence that two human commensal *Escherichia coli* isolates readily utilize ethanolamine to enhance growth, modulate gene expression, and outgrow the pathogen enterohemorrhagic *E. coli*. These data indicate a more complex role for ethanolamine in host-microbiota-pathogen interactions.

KEYWORDS ethanolamine, metabolism, microbiota, signaling

The microbiota plays essential roles in human health. For example, the microbiota functions as a barrier against invading pathogens by limiting access to nutrients (1). Significantly, bacterial pathogens have evolved to exploit specific host- and microbiota-derived metabolites to sidestep nutritional competition and control expression of virulence traits (1). For instance, ethanolamine (EA) is abundant in the gastrointestinal (GI) 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

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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 B_{12} to directly activate *eut* transcription (13, 14). Moreover, in the foodborne pathogens enterohemorrhagic *Escherichia coli* O157:H7 (EHEC) and *Salmonella enterica* serovar 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 provided as a nitrogen (but not carbon) source (Fig. 1A to C). Similarly to other *E. coli* strains, growth on EA required the addition of vitamin B_{12} (Fig. 1C).

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 NH_4 only or NH_4 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 NH_4 (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 NH_4 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 NH_4 only or NH_4 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

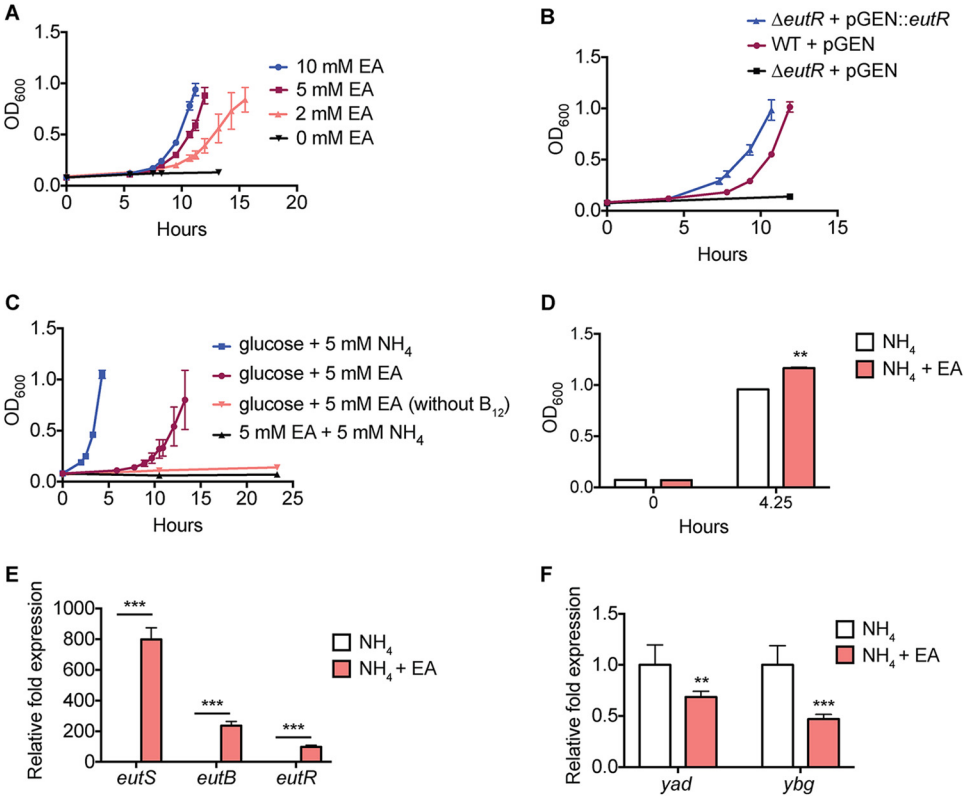

FIG 1 EA-dependent growth and signaling in *E. coli* HS. (A) Growth curve of HS grown in minimal medium with indicated EA concentrations. $n = 3$. OD_{600}, optical density at 600 nm. (B) Growth curve of wild type (WT) with empty vector, Δ*eutR* mutant with empty vector, and *eutR* complemented strain grown in minimal medium containing EA. $n = 3$. (C) Growth curve of HS grown in minimal medium with indicated carbon and nitrogen sources or without vitamin B_{12}, as specifically indicated. $n = 3$. (D) Bacterial cell density at indicated time points after growth in minimal medium with NH_4 or NH_4 and EA. $n = 3$. (E) Reverse transcription-quantitative PCR (qRT-PCR) of *eut* gene expression in HS grown in in minimal medium with NH_4 or NH_4 and EA. $n = 3$. (F) qRT-PCR of fimbrial genes in HS grown in minimal medium with NH_4 or NH_4 and EA. $n = 6$. For all, unless indicated, vitamin B_{12} was added whenever the medium was supplemented with EA. Error bars represent the mean ± standard deviation (SD). **, $P \leq 0.01$; ***, $P < 0.001$.

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 NH_4 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,

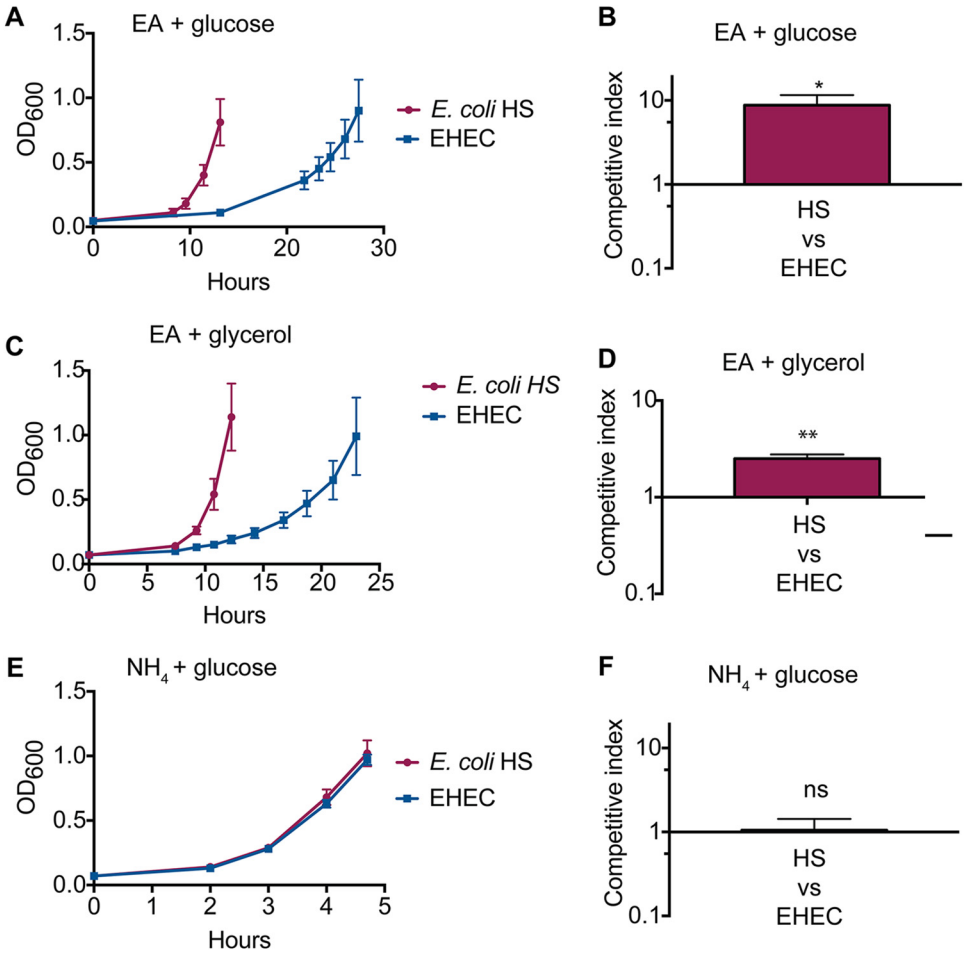

FIG 2 HS outcompetes EHEC specifically during growth on EA. (A) Growth curve of *E. coli* HS and EHEC in minimal medium with EA and glucose. (B) Competition assay between *E. coli* HS and EHEC in minimal medium with EA and glucose. (C) Growth curve of *E. coli* HS and EHEC in minimal medium with EA and glycerol. (D) Competition assay between *E. coli* HS and EHEC in minimal medium with EA and glycerol. For panels A to D, vitamin B_{12} was added to the medium. (E) Growth curve of *E. coli* HS and EHEC in minimal medium with NH_4 and glucose. (F) Competition assay between *E. coli* HS and EHEC in minimal medium with NH_4 and glucose. For all, *n* equals 3; error bars represent the mean ± standard deviation. *, $P \leq 0.05$; **, $P \leq 0.01$; ns, $P > 0.05$.

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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