From food to cell: nutrient exploitation strategies of enteropathogens

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Upon entering the human gastrointestinal tract, foodborne bacterial enteropathogens encounter, among numerous other stress conditions, nutrient competition with the host organism and the commensal microbiota. The main carbon, nitrogen and energy sources exploited by pathogens during proliferation in, and colonization of, the gut have, however, not been identified completely. In recent years, a huge body of literature has provided evidence that most enteropathogens are equipped with a large set of specific metabolic pathways to overcome nutritional limitations in vivo, thus increasing bacterial fitness during infection. These adaptations include the degradation of myo-inositol, ethanolamine cleaved from phospholipids, fucose derived from mucosal glycoconjugates, 1,2-propanediol as the fermentation product of fucose or rhamnose and several other metabolites not accessible for commensal bacteria or present in competition-free microenvironments. Interestingly, the data reviewed here point to common metabolic strategies of enteric pathogens allowing the exploitation of nutrient sources that not only are present in the gut lumen, the mucosa or epithelial cells, but also are abundant in food. An increased knowledge of the metabolic strategies developed by enteropathogens is therefore a key factor to better control foodborne diseases.

Introduction

Animals are generally considered nutrient-rich resources for micro-organisms and it is assumed that the evolution of pathogenic bacteria was at least in part driven by the opportunity to profit from increased substrate availability in host organisms (Rohmer et al., 2011). Metabolic activity is a requirement for enteric pathogens to express their full virulence as they need energy as well as carbon and nitrogen sources to proliferate within the gut, to colonize the epithelial barrier, to produce virulence factors, to withstand the host’s immune response and finally to transmit themselves to other hosts or the environment. Although the digestion activity of the intestinal microbiota and the enzymes of the host provide sufficient substrates for the indigenous gut microbes, the high nutrient competition between the commensal microbiota and enteropathogens requires the latter to occupy their own metabolic niches to survive and proliferate within the gut. As they use metabolic traits absent in avirulent bacteria, pathogens now appear to be adapted specifically to their varying environments during infection in terms of metabolism – a fact that also points to a shortage of easily available nutrients in those niches. The role of metabolism is therefore increasingly recognized as a priority equivalent to studying pathogenicity, and metabolic factors required for successful infection are considered virulence factors (Abu Kwaik & Bumann, 2013; Fuchs et al., 2012a). Consequently, the identification of key enzymes such as PyCα essential for metabolic functions during infection might open the way to novel therapeutic compounds (Schar et al., 2010).

The complex interdependencies between the gut microbiota, host and pathogens remain to be elucidated in more detail. Given the strong competition for nutrients by the resident microbiota of the gut, this host compartment provides a particular challenge for pathogens. In turn, the availability and composition of nutrients are well known to trigger the regulation of virulence factors, e.g. by carbon catabolite repression (Fuchs et al., 2012a; Le Bouguenec & Schouler, 2011; Poncet et al., 2009). Modulations of the surrounding metabolome might also activate virulence factors via the stringent response through (p)ppGpp (Dalebroux et al., 2010). It is therefore tempting to speculate that the specific metabolic adaptations of enteropathogens during infection might have been shaped by the dynamic interaction with the gut microbiota and its metabolome.

Major efforts have been made during recent years to better understand the metabolism of enteric pathogens such as...
Salmonella enterica and Listeria monocytogenes, with a focus on their intracellular replication or systemic infection (Becker et al., 2006; Eisenreich et al., 2010; Fuchs et al., 2012a, b; Schauer et al., 2010; Steeb et al., 2013). However, an overview of the metabolic strategies of pathogens during proliferation in, and colonization of, the gut is still missing. Here, we review the major nutrient requirements of foodborne enteropathogens during their life cycle with a focus on the complex environment of the gut. Examples of functional links between metabolic adaptations and bacterial virulence are also emphasized. The bacteria considered belong to the genera Bacillus, Salmonella, Shigella, Campylobacter, Listeria, Clostridium, Enterococcus, Escherichia, Yersinia and Vibrio, all of them causing gastroenteritis or related diseases. The title of this review suggests that metabolic adaptations useful in the gut also provide growth advantages prior to and during infection. To support the ‘from food to cell’ hypothesis, we provide evidence that some of the substrate degradation properties delineated below are useful not only to cope with nutrient limitations within the gut or, in the case of the facultative intracellular pathogens L. monocytogenes and Salmonella enterica, within epithelial cells, but also during proliferation in food.

**Gut microbiota**

About 1000 bacterial species inhabit the human intestine and an individual can harbour at least 160 species with up to $10^{13}$ prokaryotic cells (Qin et al., 2010). It is estimated that 50–65 g of hexose sugars are required to substitute the 15–20 g dry weight of bacteria excreted per day and individual (McNeil, 1984). The composition and diversity of the gut microbiota are variable, and reflect the maturation phases of the host as well as its diet (Dominguez-Bello et al., 2010). Metagenomic studies of the microbiome identified Bacteroidetes, Firmicutes, Actinobacteria, Fusobacteria, Lentisphaerae, Proteobacteria, Verrucomicrobia and Deinococcus–Thermus as the most common phyla in the gut microbiota, with a prevalence of the first two phyla (Maccaferrì et al., 2011; Qin et al., 2010). The proximal small intestine is only weakly colonized, and is characterized by the host digestion of carbohydrates, fat and proteins, and the absorption of monosaccharides (Martins dos Santos et al., 2010), whereas the distal regions of the gut are densely colonized. They are characterized by a low oxygen tension (Contag et al., 1995), with the exception of the mucosa as the only zone of relative oxygenation due to diffusion from the capillary network at the tips of villi (Marteyn et al., 2010). The anoxic environment supports the colonization by anaerobic bacteria, and their enzymic reservoir benefits the host with nutrients and energy by degrading otherwise indigestible polysaccharides (Backhed et al., 2005; Hooper et al., 2002; Savage, 1977).

The microbiota is also known to affect the development of the immune system, which in turn shapes the composition of the gut bacteria (Nicholson et al., 2012). The interaction between the host and its commensals is characterized by mutual benefits: whilst the host provides a warm and nutrient-rich environment, the highly adaptive metabolic activity of the commensals optimizes the nutrient and energy extraction from food (Maynard et al., 2012). Last, but not least, the intestinal microbiota is assumed to deplete most nutrients as one of several strategies that provide colonization resistance against bacterial pathogens. This theory is known as Freter’s nutrient niche hypothesis (Freter et al., 1983). For instance, commensal bacteria such as Escherichia coli strain Nissle 1917 may outcompete and reduce Salmonella enterica sv. Typhimurium colonization due to their iron uptake capacities (Deriu et al., 2013). Another example is the occupation of two distinct nutritional niches by E. coli strains HS and Nissle 1917 which together consume five sugars that are also important for the colonization of streptomycin-treated mice by pathogenic E. coli (Maltby et al., 2013). A counterpoint to the phenomenon of colonization resistance was the finding that the metabolism of bile salts by the microbiota stimulates the germination of Clostridium difficile spores (Giel et al., 2010).

**Gut metabolome**

The composition of the metabolites in the intestine is a consequence of the diet, on the one hand, and the enzymic activities of both the host and the microbiota, on the other hand. Following food ingestion, both the commensal microbiota and the host exploit food nutrients as efficiently as possible, based partially on metabolic complementarities between the two partners. Studies analysing intact human biopsies or the colonic metabolome of mice identified amino acids, amino sugars (glucosamine, galactosamine, N-acetylgalactosamine), sialic acids such as N-acetylneuraminic acid, dipeptides, carboxylic acids, pyrimidines, membrane component metabolites, urea, creatine, glucose, inositol and triglycerides to be common in the gastrointestinal tract (Matsumoto et al., 2012; Wang et al., 2007). As expected, their concentrations vary with the intestinal location. For example, the duodenum and jejunum are rich in choline, glutathione, glycero-phosphocholine (GPC), lipids and glycoproteins, but relatively poor with respect to acetate, glutamate, inositol and phosphorylcholine. The ileum is rich in amino acids, ethanolamine and glycoproteins, but poor in GPC. The transverse colonic mucosa contains higher levels of acetate, glutamate, inositol and lactate. Interestingly, a transcriptome profile during infection indicates that bacteria encounter amino acid starvation at the mucosal wall (Harvey et al., 2011).

In addition to protein-rich food, humans and animals commonly consume plant polysaccharides such as cellulose, hemicellulose, mannann, pectin or raffinose that escape absorption in the small intestine. The commensal microbiota in the distal gastrointestinal tract is able to degrade these otherwise poorly utilized macromolecules that are rich in xylose, glucose, galacturonic acid, galactose, fructose, mannose and arabinose (Hooper et al., 2002). The main chemical classes of gut metabolites contributed by the
microbiota activity are bile acids, cholines, benzoil and phenol derivatives, indoles, vitamins, polyamines, lipids and short-chain fatty acids (SCFAs) (Cummings & Macfarlane, 1991; Nicholson et al., 2012). The predominant SCFAs acetate, (2-methyl-)propionate and (iso-)butyrate are derived from pyruvate fermentation by anaerobic bacteria in the distal intestinal lumen, thus providing additional carbon and energy. The SCFAs are then mainly absorbed by the host (Hooper et al., 2002). Under a diet rich in plant polysaccharides and low in sugar or fat, the concentration of SCFAs might exceed the absorption rate (De Filippo et al., 2010). The presence of SCFAs, which are assumed to inhibit bacterial growth by decreasing the luminal pH (Cummings et al., 1987), closely links metabolism to virulence: Salmonella enterica shows limited invasion of epithelial cells in the intestine due to the specific downregulation of Salmonella pathogenicity island 1 (SPI1) genes upon exposure to butyric acid (Gantois et al., 2006), mainly synthesized by Firmicutes (Martins dos Santos et al., 2010).

The gut microbiota also provides a broad enzymic capacity to cleave host glycans or glycoconjugates such as highly glycosylated mucin or glycosphingolipids from epithelial cells. A metagenomic study comparing 18 microbiomes identified 156 families of carbohydrate-active enzymes, including glycoside hyrolases, glycosyltransferases, polysaccharide lyases and carbohydrate-esterase families (Turnbaugh et al., 2009), and the microbiome represents at least 81 different glycoside hyrolase families that are absent in the human ‘glycobiome’ (Gill et al., 2006). These data indicate the enormous capability of the gut microbiota to metabolize various carbon sources derived from ingested food or host glycoconjugates especially present on the surface of epithelial cells. An outstanding example for this metabolic capacity is Bacteroides thetaiotaomicron, a common, Gram-negative member of the intestinal microbiota in humans and mice that expresses more glycosylhydrolases than any other prokaryote sequenced so far, and it is concluded that nearly all glycosidic bonds found in nature can be cleaved by this gut bacterium (Wexler, 2007).

**Disturbances of the gut metabolome: diet, inflammation and antibiotic treatment**

The interdependencies between the host and the microbiota are complex, and their metabolic equilibrium can be disrupted by external factors. As revealed by a metagenomic approach, food profoundly affects the composition of the microbiota that is able to respond to a change in the diet within 1 day (Turnbaugh et al., 2009). A comparative study with children from Europe, with a diet rich in animal proteins, sugar and starch, and from rural Africa, with a fibre-rich, predominantly vegetarian diet, revealed an impact of dietary habits on the gut microbiota. Enterobacteriaceae such as Shigella and Escherichia in the African children were under-represented, possibly due to the abundance of SCFA-producing bacteria in their intestines (De Filippo et al., 2010). Different illnesses, such as inflammatory bowel disease, are associated with imbalances of the microbiota, called dysbiosis, which is characterized by a reduction in taxonomic diversity and species membership (Pham & Lawley, 2014; Winter & Baumler, 2014), and, consequently, by a change of the metabolic functions present in the gut lumen. Dysbiosis is also due to host-mediated inflammation upon infection and has been demonstrated to disrupt the colonization resistance, resulting in a decrease of inhibitory substances, including SCFAs, and an increase of nutrient availability within the gut (Lupp et al., 2007; Stecher et al., 2007; Stecher & Hardt, 2008). Examples for the latter are the local release of high-energy nutrients like galactose-containing glyco-conjugates as an element of the mucosal defence (McGuckin et al., 2011; Stecher et al., 2008) and the formation of tetrathionate, an alternative electron acceptor for respiration under anaerobic conditions, which allows Salmonella enterica sv. Typhimurium to use sugars more efficiently and thus to overgrow the microbiota (see below). E. coli strains that harbour nitrate reductases also benefit from intestinal inflammation as they can use nitrate as an electron acceptor to obtain a growth advantage over the majority of the microbiota (Winter et al., 2013). Nitrate is present in the intestine at physiologically relevant concentrations (Jones et al., 2007) and, like tetrathionate, it is produced upon infection, inducing the expression of nitric oxide synthetases, which generate nitric oxide. Nitric oxide can react with superoxide radicals produced during inflammation to yield peroxynitrite, which is rapidly converted to nitrate (Spees et al., 2013). Enterococcus faecalis, Bacillus cereus, Clostridium perfringens, Salmonella enterica, Shigella spp. and pathogenic E. coli possess nitrate reductases which might help these enteric pathogens to overcome colonization resistance. Thus, by triggering mucosal inflammation, these and further, yet unknown properties enable pathogens to successfully colonize the host’s intestine under otherwise adverse conditions. Inappropriate antibiotic exposure is another reason for dysbiosis that might lead to massive growth of enteric pathogens in the gut due to greater resistance to host defences and better utilization of the gut nutrient environment (Keeney & Finlay, 2011). For example, the antibiotic-associated pathogens Salmonella enterica sv. Typhimurium and Clostridium difficile profit from an increase of mucosal carbohydrates that are not further consumed by Bacteroidetes (Ng et al., 2013).

**A frugal or an opulent meal? Nitrogen, carbon and energy sources used by enteropathogens**

Despite its richness in metabolites, the gut is considered a competitive rather than a nutrient-rich environment for pathogens. All metabolic niches are already occupied by the commensal microbiota and high-energy nutrients are missing (Derrien et al., 2004; Hansson, 2012). The metabolism of pathogens in vivo has been largely unknown. A deeper understanding of this topic has been hampered by (i) the assumption that metabolism only contributes slightly to growth advantages in vivo, (ii) the absence of...
appropriate animal experiments and (iii) the finding that bacterial metabolism is robust in a sense that one pathway might easily be substituted by another. Transcriptomic studies and predictions from in vitro and bioinformatic studies, however, have now helped to overcome this lack of knowledge. Seminal progress in this field was made by establishing an infection model with streptomycin-treated mice that, in contrast to untreated mice, resembles many aspects of human enteritis, including mucosal inflammation, upon Salmonella enterica sv. Typhimurium infection (Barthel et al., 2003; Kaiser et al., 2012). This model takes advantage of the effects of dysbiosis described above. Gram-positive microbes are either unaffected by streptomycin treatment or at most reduced in numbers for only 1 day, whereas Gram-negative bacteria are completely eliminated but reappear after 1–2 days, except E. coli (Miller & Bohnhoff, 1963). Therefore, the increased susceptibility of mice for Salmonella enterica sv. Typhimurium infections in this model is probably due to the depletion of the commensal microbiota.

It is noted in the following text if mice were treated with streptomycin or used as monoassociated animals. In all other cases, e.g. in earlier studies, untreated mice were used by the authors. It should also be emphasized here that animals, in addition to many other aspects, differ from humans in terms of diet and microbiota, and that data obtained by infection models exploited in research cannot be transferred directly to humans.

Nitrogen sources

The presence of amino acids, dipeptides and oligopeptides derived from the diet or the mucosa suggests an abundance of nitrogen sources in the intestine and colon (Matsumoto et al., 2012; Zheng et al., 2011). They either are utilized directly for protein synthesis or serve as carbon and nitrogen sources following enzymic conversion. However, it is largely unknown whether or not enteric pathogens can profit from this nitrogen pool without being outcompeted by the commensal microbiota. It is well known that glutamate and glutamine are the primary nitrogen donors for all cellular metabolites in Salmonella enterica, L. monocytogenes and other enteric bacteria (Joseph et al., 2006; Klose & Mekalanos, 1997). L. monocytogenes strains lacking oligopeptide transporters are attenuated in vivo, pointing to the need for amino acid uptake and utilization (Boreeze et al., 2000; Schauer et al., 2010). Due to incomplete glycolytic, Entner–Doudoroff and pentose phosphate pathways, Campylobacter jejuni predominantly uses amino acids as the carbon and energy source instead of common sugars during infection (Parkhill et al., 2000). l-Serine, for example, is converted to pyruvate and ammonia, and a lack of this catabolic function results in a growth defect in vivo (Velayudhan et al., 2004).

Urea is generated by host tissues and passed into the gut where it is hydrolysed to ammonia by ureases of the intestinal microbiota, which thus contribute to nitrogen recycling in the intestine (Forsythe & Parker, 1985). Some of the enteropathogens discussed here, i.e. Bacillus cereus, Clostridium perfringens, Yersinia enterocolitica, Vibrio parahaemolyticus and enterohaemorrhagic E. coli (EHEC), harbour genes encoding ureolytic activities (Table 1). So far, urease has only been demonstrated to play a role in gut colonization by Shiga toxin-producing E. coli (Steyert & Kaper, 2012) and it remains to be investigated to what extent ureases contribute to improved nitrogen availability of other enteric pathogens.

Hydrogen

Molecular hydrogen is produced as a fermentation byproduct of the microbiota in the large intestine of animals, where it is removed by mesophilic methanogenic archaea (Stams, 1994). Its production can be correlated with the digestibility of the carbohydrates consumed (Maier, 2005). Interestingly, most enteropathogenic bacteria, including Campylobacter jejuni, E. coli, Y. enterocolitica, Shigella flexneri and Salmonella enterica, are able to use hydrogen as an energy source through respiratory hydrogenases (Table 1). Salmonella enterica encodes three putative membrane-associated hydrogenases, all of which are coupled to a hydrogen-oxidizing respiratory pathway which allows energy production. The Salmonella hydrogenases contribute to virulence in a typhoid fever mouse model as demonstrated by the strongly enhanced survival rate of mice infected with (multiple) hydrogenase deletion mutants of Salmonella enterica sv. Typhimurium (Maier et al., 2004). Consequently, antibiotic treatment of the mice as well as gut precolonization with a hydrogen-consuming strain depleted the initial growth advantages of Salmonella enterica using hydrogen in the gut (Maier et al., 2013). Molecular hydrogen produced by the gut microbiota was also demonstrated to support Salmonella enterica sv. Typhimurium growth with the alternative carbon source glucarate, an oxidized product of glucose, which contributes to proliferation of this pathogen in vivo (Lamichhane-Khadka et al., 2013).

Carbon and energy sources

Sugars are the most favourable energy and carbon sources for bacteria. As E. coli is a typical member of the gut microbiota, it is of interest whether or not pathogenic E. coli strains differ from commensal strains with respect to carbon and energy sources. Commensal E. coli are assumed to use a variety of mucus-derived metabolites, such as arabinose, fucose, sialic acid, N-acetylgalactosamine, glucurionate and N-acetylneuraminic acid, during replication in the gut of mice treated with streptomycin (Chang et al., 2004; Fabich et al., 2008). In the same studies, an attenuation of E. coli strains defective in the Entner–Doudoroff pathway or glycolysis in the mouse intestine was observed, whereas the glycerol, galactitol, lactose, mannitol or aspartate utilization pathways as well as the TCA cycle, the pentose phosphate pathway or gluconeogenesis were shown to be
### Table 1. Specific metabolic properties of enteropathogens

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Ethanolamine (eutABC)</th>
<th>Propanediol (pdu)</th>
<th>Cobalamin synthesis (cob/chb)</th>
<th>Tetrahydrofolate reductase (trAB, trSR)</th>
<th>5-sulfosalicylic acid (nanRATEK)</th>
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</thead>
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<tr>
<td><strong>Gram-positive</strong></td>
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<td><em>Enterococcus faecalis</em></td>
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<td><em>Bacillus cereus</em></td>
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<td>+</td>
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<tr>
<td><em>Listeria monocytogenes</em></td>
<td>+</td>
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<td><em>Clostridium perfringens</em></td>
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<td><em>Clostridium difficile</em></td>
<td>+</td>
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<td><strong>Gram-negative</strong></td>
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<td><em>Salmonella enterica</em></td>
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<td><em>Shigella flexneri</em></td>
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<td><em>Shigella dysenteriae</em></td>
<td>+</td>
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<tr>
<td><em>Shigella boydii</em></td>
<td>+</td>
<td>–</td>
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<tr>
<td><em>Shigella sonnei</em></td>
<td>+</td>
<td>–</td>
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<tr>
<td><em>Yersinia enterocolitica</em></td>
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<td>+</td>
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<td><em>Campylobacter jejuni</em></td>
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<td><em>Vibrio cholerae</em></td>
<td>–</td>
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<tr>
<td><em>Vibrio parahaemolyticus</em></td>
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<td>–</td>
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<tr>
<td><em>Escherichia coli</em></td>
<td>+</td>
<td>–</td>
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*References: 1, Almagro-Moreno & Boyd (2009a); 2, Gu et al. (2010); 3, Zhang et al. (2009); 4, Zúñiga et al. (2005); 5, Dupuy et al. (1997); 6, Barrett & Clark (1987); 7, Kröger & Fuchs (2009).*(nanA, N-acytelyneuraminic lyase; nanE, N-acetylmannosamine 6-phosphate epimerase; nanK, N-acetylmannosamine kinase.
+ , Genes present; –, genes absent; bold, in vivo relevance has been demonstrated (see text for details).

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+ , Genes present; –, genes absent; bold, in vivo relevance has been demonstrated (see text for details).

*nanA, N-acytelyneuraminic lyase; nanE, N-acetylmannosamine 6-phosphate epimerase; nanK, N-acetylmannosamine kinase.
+ , Genes present; –, genes absent; bold, in vivo relevance has been demonstrated (see text for details).

*Cobalamin-dependent glycerol dehydratase; anaerobic degradation of glycerol (Lawrence & Roth, 1996).
dispensable. In contrast, pathogenic *E. coli* strain O157:H7 does not use gluconate and *N*-acetylneuraminic acid, but does use galactose, hexonurates, mannose and ribose. Mutants of *E. coli* O157:H7 defective in the utilization of fucose, but not of *N*-acylgalactosamin, showed a weaker colonization of adult cattle treated with streptomycin (Snider et al., 2009).

During proliferation within the gut, *L. monocytogenes* specifically profits from the uptake of sugars and their alcohols, including mannitol, mannose, galactitol and fructose, as indicated by the increased transcription of the respective phosphoenolpyruvate-dependent phosphotransferase system (PTS) (Toledo-Arana et al., 2009). The same study identified the upregulation of genes encoding dihydroxyacetone kinases involved in glycerol utilization, which is already known to play an important role during intracellular replication of this pathogen (Eylert et al., 2008; Joseph et al., 2006). Upon entering epithelial cells, *L. monocytogenes* also upregulates mannose- and fructose-specific PTGs (Chatterjee et al., 2006; Joseph et al., 2006). The main virulence regulator of *L. monocytogenes*, PrfA, is downregulated in the presence of efficiently utilized sugars such as glucose, indicating that these carbon sources are limited when this facultative intracellular pathogen starts to invade epithelial cells (Behari & Youngman, 1998). In EHEC, glycolytic conditions similarly inhibit the expression of *ler* and thus of all other LEE (locus of enterocyte effacement) genes (Njoroge et al., 2012).

As indicated by transcriptomic studies using the enteritis infection model, the uptake of ribose, arginine and glycerol is important for the proliferation of *Salmonella enterica* in the caecal lumen of chicken or mice (Harvey et al., 2011; Rollenhagen & Bumann, 2006). The same studies suggest that *Salmonella* spp. use nutrients such as propionate, melibiose, trehalose and *L*-ascorbate during replication in the intestine. Loss of the ability to degrade propionate, e.g. the lack of *mhp* genes in *Shigella* spp. and in enterovasive *E. coli*, has been interpreted as an adaptation to the intracellular life cycle of these pathogens, especially because the degradation product 2-methylcitrate inhibits gluconeogenesis critical for *E. coli* virulence (Alteri et al., 2009; Rohmer et al., 2011). *D*-Xylose is derived from xylan and xyloglucan, the major hemicellulose component of plant cell walls, and can be utilized via *D*-xylulose by most of the enteropathogens discussed here with the exception of *Clostridium perfringens* and *L. monocytogenes* (Table 1).

Thus, pathogens benefit from the simultaneous exploitation of several sugars that are probably not utilized by the commensal intestinal microbiota. An exception is *Bacteroides thetaiotaomicron* that consumes a similar set of carbohydrates, among them the four main components of mucin, i.e. galactose, *N*-acylgalactosamine, *N*-acylgalactosamine and fucose (Salyers & Pajau, 1989), instead of polysaccharides accidentally absent in the diet (Sonnenburg et al., 2005). The role of the mucus for the metabolism of enteric pathogens remains to be further elucidated.

**Mucus-derived nutrients and the proliferation of commensal and pathogenic bacteria**

In addition to the lumen, other relevant gut compartments encountered by pathogenic bacteria are the mucus-associated space and the mucus. The mucus layer protects the underlying cells against mechanical damage, assists food particle absorption, separates epithelial cells and bacteria of the gut lumen, and, last but not least, constitutes a barrier for pathogens (Corazziari, 2009). The water-rich mucus consists of mucins composed of oligosaccharides (glycans) attached to a protein backbone. The outer mucus layer of the stomach and colon is, in contrast to the inner mucus layer, penetrated by commensal bacteria (Johansson et al., 2008). More than 100 different mucin oligosaccharides have been identified, with a remarkable variation of glycosylation within the various mucin populations of the intestinal regions (Robbe et al., 2004). Mucin carbohydrate side-chains mainly contain galactose, hexosamines such as *N*-acylgalactosamine, and fucose (Cummings & Macfarlane, 1991). At the mucosal wall, sugars such as mannose, galactose, fucose and glucose are available as components of mucin glycoconjugates (Juge, 2012). Fucose is known to be degraded by commensal bacteria including *Bifidobacteria* and *Bacteroides* (Gill et al., 2006; Hooper et al., 1999). *Bacteroides thetaiotaomicron* is even able to induce the production of fucosylated glycoconjugates in the distal intestine – an ability that requires a certain population density of the bacterium (Becker & Lowe, 2003; Bry et al., 1996). The spatio-temporal dynamic of epithelial fucosylation confers a growth advantage to *Bacteroides thetaiotaomicron* and provides favourable or unfavourable colonization conditions for other commensals as well as pathogens, thus modifying the composition of the gut microbiota (Hooper et al., 2002).

Exploiting microbiota-released sugars of the epithelial glycocalyx appeared as a successful strategy of enteropathogens to overcome nutrient limitations when proliferating within or passing through this barrier to the surface of the epithelial cells (Gill et al., 2006; Ng et al., 2013; Stahl et al., 2011). Obvious advantages of glycan utilization are that glycans are replenished continuously, and readily fermented as carbon and energy source (Salyers & Pajau, 1989). A chemotactic gradient of *D*-galactose, but not *L*-fucose, was found to lead *Salmonella* from the lumen to the mucosal surface (Stecher et al., 2008). It can be assumed that other gastrointestinal pathogens are attracted by similar chemotactic gradients towards the cell surface. Moreover, mutations in flagellar assembly and chemotaxis impaired the fitness of *Salmonella enterica* sv. Typhimurium in the inflamed, but not the healthy, intestine of specific pathogen-free mice (Stecher et al., 2008). This phenomenon is likely caused by a *Salmonella enterica* sv. Typhimurium-induced inflammation which results in the enhanced secretion of mucus by epithelial cells, thus providing further nutrients for *Salmonella enterica* sv. Typhimurium (McGuckin et al., 2011). Consequently, this pathogen grows well in caecal
mucus, but not in caecal luminal contents of streptomycin-treated mice (McCormick et al., 1988).

**L-Fucose, L-rhamnose and sialic acid**

L-Fucose and L-rhamnose are components of the carbohydrate moiety of the mucosal glycoconjugates. Additionally, they can be frequently found in plant cell wall polysaccharides and in bacterial exopolysaccharides (Sampson & Bobik, 2008). α-Linked L-fucose represents 4–14% of the total oligosaccharide content of mucin and is one of the terminal sugars of the oligosaccharide chains attached to the protein backbone of mucins (Muraoka & Zhang, 2011). Both sugars are derived from ingested food or from shedding and turnover of epithelial cells, and at least fucose is abundant in the intestine (Robbe et al., 2004). L-Fucose is also present in human milk oligosaccharides (below) and the monosaccharide rhamnose is a building block of pectin. Sialic (or neuraminic) acid is the only component apart from L-fucose that is uniquely associated with mucin (Salyers & Pajeau, 1989).

**Factors involved in fucose degradation**

In *E. coli*, L-rhamnose induces the *rha* system, which results in the degradation of L-rhamnose to l-lactaldehyde. The *rha* system consists of the enzymes RhaD (rhamnulose 1-phosphate aldolase), RhaA (L-rhamnose isomerase), RhaB (L-rhamnulose kinase) and RhaC (regulatory protein for *rhaDAB*) (Power, 1967). Expression of 1,2-propanediol oxidoreductase (*FucO*) is induced when *E. coli* grows anaerobically on L-rhamnose, converting l-lactaldehyde to lactate under aerobic conditions, were first shown to accumulate L-fucose 1-phosphate show stimulation of ribose utilization, indicating the cross-regulation of unrelated carbon sources under nutritional depletion (Alteri & Mobley, 2012). *L. monocytogenes* strains and *Clostridium perfringens* are also equipped with the *fuc* genes, whereas most *Vibrio cholerae* strains and the pathogenic *Yersinia* spp. probably lack the capability to degrade fucose (Table 1). Deletion of genes involved in fucose utilization by *Salmonella enterica* reduces its competitiveness in vivo (Ng et al., 2013). *E. coli* accumulating fucose 1-phosphate show stimulation of ribose utilization, indicating the cross-regulation of unrelated carbon sources under nutritional depletion (Alteri & Mobley, 2012). *L. monocytogenes* and *Bacillus cereus* encode a fucose isomerase, but further genes of these Gram-positive enteropathogens involved in fucose utilization have not been annotated. However, the *Bacillus cereus* genes flanking *fucI* as well as their homologues in the genome of *L. monocytogenes* encode enzymes with putative functions in sugar metabolism or regulation and transport.

**L-Rhamnose and L-fucose utilization by enteric pathogens**

Although the capability to degrade L-rhamnose is present in the genomes of many enteropathogens (Table 1), a role of this metabolic pathway *in vivo* remains to be elucidated. Recently, a novel transcription factor was identified in *L. monocytogenes* strains associated with foodborne infections and it could be demonstrated that this *prfA*-like regulator contributes to L-rhamnose utilization (Salazar et al., 2013).

The ability of some enteropathogens to degrade fucose has been demonstrated experimentally (Badia et al., 1985; Obradors et al., 1988) or predicted due to genome analyses. As mentioned above, *Campylobacter jejuni* is unable to use common carbohydrates as a carbon or energy source, although all genes required for gluconeogenesis as well as an intact TCA cycle are present in the genome (Parkhill et al., 2000). However, fucose derived from fucosylated glycoconjugates on the surface of epithelial cells was discovered recently to serve as a carbon and energy source of *Campylobacter jejuni*, and strains lacking the capability to use fucose are attenuated *in vivo* (Stahl et al., 2011). In addition to mucin, organic acid intermediates of the TCA cycle as well as some amino acids, L-fucose was identified as a chemoattractant for *Campylobacter jejuni* (Hugdahl et al., 1988). *Campylobacter jejuni* binds to 1,2-α-fucosylated glycans *in vitro* and adherence to intestinal cells is diminished by free fucose (Muraoka & Zhang, 2011). Similar to *Bacteroides thetaiotaomicron*, the anaerobic utilization of fucose by *Campylobacter jejuni* results in pyruvate production (Stahl et al., 2012). The exact mechanisms of the L-fucose degradation pathway in *Campylobacter jejuni* remain to be elucidated, since only a fucose permease as well as an aldolase homologue of *E. coli*, which converts l-lactaldehyde to lactate under aerobic conditions, were identified to be involved in fucose degradation by *Campylobacter jejuni* (Stahl et al., 2011).

The genomes of *Salmonella enterica*, pathogenic *E. coli* strains and *Clostridium perfringens* are also equipped with the *fuc* genes, whereas most *Vibrio cholerae* strains and the pathogenic *Yersinia* spp. probably lack the capability to degrade fucose (Table 1). Deletion of genes involved in fucose utilization by *Salmonella enterica* reduces its competitiveness in vivo (Ng et al., 2013). *E. coli* accumulating fucose 1-phosphate show stimulation of ribose utilization, indicating the cross-regulation of unrelated carbon sources under nutritional depletion (Alteri & Mobley, 2012). *L. monocytogenes* and *Bacillus cereus* encode a fucose isomerase, but further genes of these Gram-positive enteropathogens involved in fucose utilization have not been annotated. However, the *Bacillus cereus* genes flanking *fucI* as well as their homologues in the genome of *L. monocytogenes* encode enzymes with putative functions in sugar metabolism or regulation and transport.

Fucose-specific glycosylhydrolases (fucosidases) as homologues of the 1,3/4-α-fucosidase from *Bifidobacterium longum* can be found in *Clostridium perfringens*, but not in *L. monocytogenes*. *Bacillus cereus* secretes an α-1-fucosidase (Miura et al., 2005). For Gram-negative enteropathogens, a protein sequence of an α-1-fucosidase of *Bacteroides thetaiotaomicron* (GenBank accession number NP_811105)
was used here for database query, but no homologues of this sequence were found in *Salmonella*, *Yersinia* or *Vibrio*. This finding suggests that these bacteria benefit from the fucose-releasing activity of commensal bacteria such as *Bacteroides thetaiotaomicron*.

The cleavage of fucose by *Bacteroides thetaiotaomicron* modulates the pathogenicity of EHEC. Using an infant rabbit infection model, it was demonstrated recently that a novel two-component-system (TCS) of this pathogen, FusKR, senses fucose. The genes encoding this TCS are located within the EHEC pathogenicity island LEE; FusK sensing results not only in the activation of the fucose utilization pathway, but also in enhanced transcription of several LEE genes involved in attaching and effacing lesions on enterocytes (Pacheco *et al.*, 2012).

**L-Fucose in milk and impact on newborn gut microbiota**

Human milk oligosaccharides (HMOs) are composed of the five monosaccharides D-glucose, D-galactose, N-acetylg glucosamine, N-acetylneuraminic acid and L-fucose. These building blocks contribute to the formation of >130 different HMOs identified so far (Bode, 2006). Fucosylation occurs frequently on the non-reducing termini of the HMOs. Due to their complex structures containing α- and β-linkages, HMOs are hydrolysed poorly by typical enterobacteria and are additionally resistant against low pH in the stomach. The high density of bifidobacteria in the intestines of breast-fed infants indicates the ability of these bacteria to hydrolyse the HMOs by removal of the 1,2-, 1,3-α- and 1,4-α-fucosyl residues (Sela *et al.*, 2008). Three fucosidases with this function have been characterized in...
Bifidobacterium bifidum (Ashida et al., 2009; Katayama et al., 2004). The breakdown of HMOs to SCFAs lowers the gut pH and thus diminishes the growth of harmful bacteria. Another health-beneficial feature of HMOs is the structural similarity of the non-reducing ends of HMOs and the non-reducing ends of glycoconjugates which are often used by pathogenic bacteria and viruses to recognize cell surface receptors and to bind to or invade target cells (Bode, 2006; Kobata, 2010). For example, Campylobacter jejuni binds to the fucosylated H-2 antigen expressed on the surface of gut epithelial cells. Interestingly, this attachment is inhibited specifically by fucosylated oligosaccharides present in HMOs (Ruiz-Palacios et al., 2003).

Sialic acid

Sialic acid encompasses a variety of nine-carbon amino sugars that are found mainly in glycoconjugates of eukaryotic cells, and contribute to cell–cell interactions and cell–molecule recognition (Varki, 1993). Several bacterial pathogens, among them Campylobacter jejuni and EHEC, sialylate their outer membrane surface to hide themselves from the host immune response (Severi et al., 2007). However, it has been demonstrated that E. coli, Clostridium perfringens and Bacteroides fragilis are able to utilize sialic acid as a carbon, energy and nitrogen source via the factors encoded by the nanRATEK operon. A bioinformatic approach identified the genes required for this catabolism additionally in Salmonella enterica, Shigella spp., V. cholerae, V. parahaemolyticus, Y. enterocolitica and Clostridium difficile (Almagro-Moreno & Boyd, 2009b), but not in Enterococcus faecalis, Bacillus cereus or L. monocytogenes. As sialic acid is found primarily in glycoconjugates, it might be hypothesized that its utilization helps gut pathogens to colonize the colon, to occupy a nutrient-rich and competitor-free niche, and/or to penetrate the mucus towards the epithelial cells (Chang et al., 2004). Indeed, it was demonstrated that the presence of SPI1 positively effects the induction of the nanRATEK utilization genes of Salmonella enterica (Golding et al., 2007), although a sialic acid transporter mutant did not reduce virulence significantly (Steeb et al., 2013). However, a lack of sialic acids due to the colonization of gnotobiotic mice with a sialidase-deficient mutant of Bacteroides thetaiotaomicron impaired the expansion of Salmonella enterica and Clostridium difficile (Ng et al., 2013).

Ethanolamine and 1,2-propanediol

The foodborne pathogens Clostridium perfringens, Salmonella enterica and L. monocytogenes are the only bacteria from Table 1 that are able to degrade ethanolamine as well as 1,2-propanediol (Korbel et al., 2005) derived from phospholipids or fucos. All other enteric pathogens lack one or both of these degradation pathways, which are also missing in the commensal Bacteroides thetaiotaomicron. The utilization of ethanolamine and 1,2-propanediol under anoxic conditions has been studied in most detail for Salmonella enterica (Roth et al., 1996). These pathways, which are summarized in Fig. 1, need exogenous tetrathionate as an alternative electron acceptor (Price-Carter et al., 2001) and the cofactor cobalamin that can be synthesized by the three pathogens (see below for details) (Price-Carter et al., 2001; Roth et al., 1996). Enteropathogens that lack the complete cobalamin synthesis apparatus either contain the BtuFCD importer or use precursors for the final production of this cofactor (Table 1).

The organization of the genes responsible for the degradation of 1,2-propanediol and ethanolamine in Salmonella enterica, L. monocytogenes and Clostridium perfringens is depicted in Fig. 2. In Salmonella enterica, the two gene sets plus the genetic determinants required for cobalamin synthesis (cob/cbi) cover ~1% of the genome and are found on two separated chromosomal fragments. In L. monocytogenes, these genes are organized consecutively in one chromosomal region, but they are scattered over the chromosome of Clostridium perfringens.

Recent publications provide indirect and direct experimental evidence for the assumption that the utilization of ethanolamine and/or 1,2-propanediol is linked closely to intestinal proliferation and thus to the virulence of L. monocytogenes, enteropathogenic E. coli (EPEC), Salmonella enterica and Enterococcus faecalis (Becker et al., 2006; Harvey et al., 2011; Kendall et al., 2012; Klumpp & Fuchs, 2007; Maadani et al., 2007; Thiennimitr et al., 2011; Toledo-Arana et al., 2009). Two virulence regulators of Salmonella, CraF and Fis, are known to control eut and pdu genes, thus further linking these metabolic pathways with infection (Kelly et al., 2004; Lawhon et al., 2003).

Genetics of ethanolamine utilization

Sequenced genomes of 84 bacteria belonging to different phyla (Proteobacteria, Firmicutes, Actinobacteria, Acidobacteria, Bacteroidetes, Fusobacteria and Chlorophlexi) were shown to harbour genes for ethanolamine utilization (eut) (Tsuy et al., 2009). In addition to Listeria, Salmonella, Shigella and Clostridium, the commensal and opportunistic pathogenic Enterococcus faecalis as well as pathogenic E. coli are equipped with the eut genes. The eut gene cluster varies in size between the phylogenetic groups: the longer eut operon, which is present in Firmicutes and Enterobacteriaceae (Tsuy et al., 2009), contains the genes eutKLMNS, whose products constitute an organelle-like structure or bacterial microcompartment reminiscent of carboxysomes (Kofoid et al., 1999; Penrod & Roth, 2006). These microcompartments are formed to optimize batched reactions by the spatial vicinity of substrates and enzymes, to protect the cell from damage from toxic intermediates such as acetaldehyde and to retain volatile intermediates (Garsin, 2010). A more effective utilization of ethanolamine may thus be especially important for pathogenic bacteria that live in competition with the commensal microbiota and are deprived of other energy sources. In contrast, Actinobacteria and most Proteobacteria have a short version of the eut operon containing only eutB and eutC, encoding the ethanolamine ammonia lyase subunits.
Genes coding for proteins of the eut cluster were found in Yersinia species avirulent towards humans, but not in the pathogenic representatives of this genus, Yersinia pseudotuberculosis and Yersinia pestis (Chen et al., 2010). In contrast to Y. enterocolitica, the systemic pathogens Y. pseudotuberculosis, Salmonella enterica sv. Typhi and Salmonella enterica sv. Paratyphi have not acquired or have lost the tetrathionate respiration pathway, presumably because they would not benefit significantly from tetrathionate reduction during extracellular growth. The putative loss of such a metabolic property is often interpreted as a consequence of the adaptation to a non-enteric life cycle, indicating that the energy burden of maintaining the respective chromosomal fragment exceeds the benefit for these pathogens (Rohmer et al., 2011). In Enterococcus faecalis, eut gene expression is regulated via a TCS (EutVW) (Garsin, 2010). In this bacterium, the availability of cobalamin may be noticed by a riboswitch identified in the untranslated region of the eut operon (Fox et al., 2009). In Salmonella enterica, the eut gene cluster (and also the cbi/eti cluster) are regulated positively by EutR, which is induced by the presence of ethanolamine and cobalamin (Sheppard & Roth, 1994).

Ethanolamine utilization in vivo

The host diet as well as the bacterial and epithelial cells in the intestine are abundant sources of ethanolamine, as phosphatidylethanolamine is a membrane lipid constituent in both mammalian and bacterial cell membranes. The highest concentrations of ethanolamine were found in the duodenum of mice, which were untreated or germ-free (Martin et al., 2009). Ethanolamine can be readily broken down to ethanolamine and 1,2-diacyl-sn-glycerol by phosphatidylethanolamine hydrolases, and serves as a carbon, nitrogen and energy source (Garsin, 2010). Phospholipases specific for phosphatidylethanolamine are present in mammal tissue, but bacterial counterparts have not been identified so far, although broad-range phospholipases such as PlcB from L. monocytogenes might exhibit such an activity. The eut genes of Salmonella enterica sv. Typhimurium are upregulated in the intestine of chicken (Harvey et al., 2011) and an eut mutant is attenuated in macrophages as well as in mice when administered per os, but not intraperitoneally (Stojiljkovic et al., 1995), pointing to the role of ethanolamine utilization during proliferation in the gut. Similar findings were made for L. monocytogenes, which upregulates the eut genes in the intestine of L. monocytogenes, Clostridium perfringens

\[\text{cob} \quad \text{cbi} \quad \text{pdu} \quad \text{eut} \]

**Fig. 2.** Genetic organization of eut, pdu and cbi genes in Salmonella enterica sv. Typhimurium LT2. The genomes of most Salmonella enterica serovars carry intact operons with most of the genes homologous to each other (genes depicted in black). The cob and pdu operons in the Salmonella enterica sv. Gallinarum strain 287/91 genome lack the genes cbiO/K/D/C and pocR, indicated in white. The organization of the eut operon within most Salmonella serovars is also shown. An exception is the eut operon of Salmonella enterica sv. Choleraesuis strain SC-B67 lacking eutA/C/K. Grey genes indicate gene replacement with respect to the LT2 genome.
<table>
<thead>
<tr>
<th>Gene</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1,2-Propanediol utilization</strong></td>
<td></td>
</tr>
<tr>
<td>cobD</td>
<td>1-Threonine O-3-phosphate decarboxylase</td>
</tr>
<tr>
<td>eutJ</td>
<td>Unknown</td>
</tr>
<tr>
<td>pduA</td>
<td>Polyhedral body component</td>
</tr>
<tr>
<td>pduB</td>
<td>Polyhedral body component</td>
</tr>
<tr>
<td>pduC</td>
<td>Diol dehydratase large α subunit</td>
</tr>
<tr>
<td>pduD</td>
<td>Diol dehydratase medium β subunit</td>
</tr>
<tr>
<td>pduE</td>
<td>Diol dehydratase small γ subunit</td>
</tr>
<tr>
<td>pduF</td>
<td>Propanediol diffusion facilitator</td>
</tr>
<tr>
<td>pduG</td>
<td>Diol dehydratase reactivation factor large subunit</td>
</tr>
<tr>
<td>pduH</td>
<td>Diol dehydratase reactivation factor small subunit</td>
</tr>
<tr>
<td>pduJ</td>
<td>Polyhedral body component</td>
</tr>
<tr>
<td>pduK</td>
<td>Polyhedral body component</td>
</tr>
<tr>
<td>pduL</td>
<td>Phosphotransacylase</td>
</tr>
<tr>
<td>pduM</td>
<td>Unknown</td>
</tr>
<tr>
<td>pduN</td>
<td>Polyhedral body component</td>
</tr>
<tr>
<td>pduO</td>
<td>Adenosyltransferase</td>
</tr>
<tr>
<td>pduP</td>
<td>Propionaldehyde dehydrogenase</td>
</tr>
<tr>
<td>pduQ</td>
<td>Propanol dehydrogenase</td>
</tr>
<tr>
<td>pduS</td>
<td>NADH:ubiquinone oxidoreductase</td>
</tr>
<tr>
<td>pduT</td>
<td>Polyhedral body component</td>
</tr>
<tr>
<td>pduU</td>
<td>Polyhedral body component</td>
</tr>
<tr>
<td>pduV</td>
<td>Unknown</td>
</tr>
<tr>
<td>pduW</td>
<td>Propionate kinase</td>
</tr>
<tr>
<td>pduX</td>
<td>1-Threonine kinase</td>
</tr>
<tr>
<td>pocR</td>
<td>Transcriptional activator of pdu operon</td>
</tr>
<tr>
<td><strong>Ethanolamine utilization</strong></td>
<td></td>
</tr>
<tr>
<td>eat</td>
<td>Ethanolamine permease</td>
</tr>
<tr>
<td>eutA</td>
<td>Reactivating factor</td>
</tr>
<tr>
<td>eutB</td>
<td>Ethanolamine ammonia lyase, large subunit</td>
</tr>
<tr>
<td>eutC</td>
<td>Ethanolamine ammonia lyase, small subunit</td>
</tr>
<tr>
<td>eutD</td>
<td>Phosphotransacylase</td>
</tr>
<tr>
<td>eutE</td>
<td>Aldehyde oxidoreductase</td>
</tr>
<tr>
<td>eutG</td>
<td>Alcohol dehydrogenase</td>
</tr>
<tr>
<td>eutH</td>
<td>Ethanolamine transport protein</td>
</tr>
<tr>
<td>eutJ</td>
<td>Putative chaperonin</td>
</tr>
<tr>
<td>eutK</td>
<td>Carboxysome structural protein</td>
</tr>
<tr>
<td>eutL</td>
<td>Carboxysome structural protein</td>
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<tr>
<td>eutM</td>
<td>Carboxysome structural protein</td>
</tr>
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<td>eutN</td>
<td>Carboxysome structural protein</td>
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<tr>
<td>eutP</td>
<td>Ethanolamine utilization protein</td>
</tr>
<tr>
<td>eutQ</td>
<td>Ethanolamine utilization protein</td>
</tr>
<tr>
<td>eutR</td>
<td>Transcriptional regulator</td>
</tr>
<tr>
<td>eutS</td>
<td>Carboxysome structural protein</td>
</tr>
<tr>
<td>eutT</td>
<td>Corrinoid cobalamin adenosyltransferase</td>
</tr>
<tr>
<td>eutV</td>
<td>Sensor histidine kinase</td>
</tr>
<tr>
<td>eutW</td>
<td>Response regulator</td>
</tr>
<tr>
<td><strong>Cobalamin synthesis</strong></td>
<td></td>
</tr>
<tr>
<td>cbmA</td>
<td>Cobyric acid A,C-diamide synthase</td>
</tr>
<tr>
<td>cbIB</td>
<td>Cobalamin biosynthesis protein</td>
</tr>
<tr>
<td>cbIC</td>
<td>Precorrin-8X methylmutase</td>
</tr>
<tr>
<td>cbID</td>
<td>Cobalt-precorrin-6A synthase</td>
</tr>
<tr>
<td>cbIE</td>
<td>Cobalt-precorrin-6Y C(5) methyltransferase</td>
</tr>
<tr>
<td>cbIT</td>
<td>Cobalt-precorrin-6Y C(15) methyltransferase</td>
</tr>
<tr>
<td>cbIF</td>
<td>Precorrin methylase</td>
</tr>
<tr>
<td>cbIG</td>
<td>Cobalamin biosynthesis protein</td>
</tr>
</tbody>
</table>
The expression of \( \text{pdu} \) and the genes for cobalamin synthesis seems to be regulated in a coordinated manner by the DNA-binding protein PocR, whose activity depends on the presence of 1,2-propanediol (Rondon et al., 1995). In this enteropathogen, the expression of \( \text{pdu} \) and the genes for cobalamin synthesis also considered to be a barrier to cobalamin, as mutants unable to form microcompartments grew faster with limiting cobalamin compared with the WT (Cheng et al., 2011). Glutathione is necessary for maximal transcription of \( \text{cobi/pdu} \) when \( \text{Salmonella enterica} \) sv. Typhimurium grows aerobically, possibly by protecting proteins in the microcompartment from damage by aldehydes (Rondon et al., 1995). In this enteropathogen, the expression of \( \text{pdu} \) and the genes for cobalamin synthesis also seems to be regulated in a coordinated manner by the DNA-binding protein PocR, whose activity depends on the presence of 1,2-propanediol (Rondon & Escalante-Semerena, 1992, 1996). In addition to \( \text{Salmonella enterica} \), the gene clusters for 1,2-propanediol utilization (\( \text{pdu} \)) and the synthesis of cobalamin (\( \text{cobi} \)) (Fig. 2) are also found in the genomes of the foodborne pathogens \( \text{L. monocytogenes} \), \( \text{Clostridium perfringens} \), \( \text{Shigella sonnei} \), EPEC, enterotoxigenic \( E. coli \) (ETEC) and \( Y. enterocolitica \) (Buchrieser et al., 2002). The microcompartment is also present in the genomes of \( L. monocytogenes \) and \( Y. enterocolitica \) (Buchrieser et al., 2002), but not in \( \text{Clostridium spp.} \), and its upregulation was observed during intracellular replication of \( L. monocytogenes \). The expression of \( \text{pdu} \) and \( \text{cobi} \) in \( \text{Clostridium} \) is assumed to be regulated by a TCS, thus reminding us of \( \text{cut} \) gene control in \( \text{Firmicutes} \). Fusobacterium nucleatum (Karpathy et al., 2007) and commensal micro-organisms such as \( \text{Roseburia inulinivorans} \) and \( \text{Propionibacterium freudenreichii} \) also harbour the \( \text{pdu} \) and \( \text{cobi} \) gene clusters. Fusose stimulates the expression of the genes involved in fusose and propanediol utilization in \( \text{R. inulinivorans} \), resulting in the formation of propionate and propanol (Scott et al., 2006). In contrast to

**Table 2. cont.**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{cbiH} )</td>
<td>Precorrin-3B C(17) methyltransferase</td>
</tr>
<tr>
<td>( \text{cbiJ} )</td>
<td>Cobalt-precorrin-6X reductase</td>
</tr>
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<td>( \text{cbiK} )</td>
<td>Cobalt chelatase</td>
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<td>( \text{cbiL} )</td>
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<td>( \text{cbiN} )</td>
<td>Cobalt transport protein</td>
</tr>
<tr>
<td>( \text{cbiO} )</td>
<td>Cobalt transport protein</td>
</tr>
<tr>
<td>( \text{cbiP} )</td>
<td>Cytochrome c oxidase</td>
</tr>
<tr>
<td>( \text{cobi} )</td>
<td>Cobalt-dependent 1,2-propanediol dehydratase</td>
</tr>
<tr>
<td>( \text{cobi} )</td>
<td>Cobalamin transport over outer membrane</td>
</tr>
<tr>
<td>( \text{cobiF} )</td>
<td>Cobalamin transport over outer membrane</td>
</tr>
<tr>
<td>( \text{cobiG} )</td>
<td>Cobalamin transport over inner membrane</td>
</tr>
<tr>
<td>( \text{btuFCD} )</td>
<td>Cobalamin transport over inner membrane</td>
</tr>
<tr>
<td>( \text{btuB} )</td>
<td>Cobalamin transport over outer membrane</td>
</tr>
<tr>
<td>( \text{btuB} )</td>
<td>Cobalamin transport over outer membrane</td>
</tr>
</tbody>
</table>

**Utilization of 1,2-propanediol: distribution, regulation and in vivo relevance**

1,2-Propanediol is the product of bacterial growth on fusose and/or rhamnose derived from mucosal glycoconjugsates. In \( \text{Salmonella enterica} \), eight genes of the \( \text{pdu} \) operon (Table 2) encode shell proteins of another microcompartment which might protect the cell from the toxic intermediate propionaldehyde, which is formed by cobalamin-dependent 1,2-propanediol dehydratase (\( \text{pduCDE} \)) and further processed to propanol or propionyl-coenzyme A (Havemann et al., 2002). The microcompartment is also present in the genomes of the foodborne pathogens \( \text{L. monocytogenes} \), \( \text{Clostridium perfringens} \), \( \text{Shigella sonnei} \), EPEC, enterotoxigenic \( E. coli \) (ETEC) and \( Y. enterocolitica \) (Buchrieser et al., 2002). The microcompartment is also present in the genomes of \( L. monocytogenes \) and \( Y. enterocolitica \) (Buchrieser et al., 2002), but not in \( \text{Clostridium spp.} \), and its upregulation was observed during intracellular replication of \( L. monocytogenes \). The expression of \( \text{pdu} \) and \( \text{cobi} \) in \( \text{Clostridium} \) is assumed to be regulated by a TCS, thus reminding us of \( \text{cut} \) gene control in \( \text{Firmicutes} \). Fusobacterium nucleatum (Karpathy et al., 2007) and commensal micro-organisms such as \( \text{Roseburia inulinivorans} \) and \( \text{Propionibacterium freudenreichii} \) also harbour the \( \text{pdu} \) and \( \text{cobi} \) gene clusters. Fusose stimulates the expression of the genes involved in fusose and propanediol utilization in \( \text{R. inulinivorans} \), resulting in the formation of propionate and propanol (Scott et al., 2006). In contrast to
Salmonella, dehydration of the intermediate 1,2-propanediol involves a cobalamin-independent enzyme of R. inulinivorans.

The pdu operon is involved in pathogenicity of Salmonella enterica sv. Typhimurium, as the removal of the region resulted in a virulence defect (Conner et al., 1998; Heitloff et al., 1999). The pdu genes are also upregulated during growth in macrophages, epithelial tissue cells and in the gut of chicken (Harvey et al., 2011; Hautefort et al., 2008). Indeed, a Salmonella enterica sv. Typhimurium propanediol utilization mutant shows attenuated growth in mice macrophages (Klump & Fuchs, 2007). Therefore, when pathogenic micro-organisms such as Listeria, Clostridium, Salmonella, Shigella and Yersinia occupy anaerobic habitats such as the mammalian gut, the anaerobic degradation of 1,2-propanediol may provide a benefit over other bacteria not able to use such fermentation end products.

Inositol

Another example of the metabolic adaptation of enteric pathogens is the utilization of myo-inositol – a polyol that is abundant in soil. Its phosphorylated form, inositol hexakisphosphate or phytate, serves as a phosphorus store in plants. It can be utilized by livestock in the presence of phytases which are present in many representatives of the resident microflora, such as Bifidobacterium pseudocatenulatum (Haros et al., 2009). Phytic acid is naturally present in whole cereal grains, seeds, nuts and legumes, with up to 3–6% phytic acid (Reddy, 2001). The daily intake of phytate was estimated to be 2000–2600 mg for vegetarian diets and 150–1400 mg for mixed diets (Reddy, 2001). Similar to ethanolamine, the duodenum has a higher content of myo-inositol than the other intestinal tissues (Martin et al., 2009). Strikingly, the ability to grow with myo-inositol is found in the Gram-positive enteropathogens Enterococcus faecalis, Bacillus cereus, L. monocytogenes and Clostridium perfringens, all of which are found commonly in soil. Utilization of myo-inositol as a sole carbon and energy source by Salmonella enterica sv. Typhimurium in vitro has been characterized recently (Kröger & Fuchs, 2009). All iol genes involved in this degradation pathway are localized on a genomic island (GEI4417/4436) that has so far been found in the genomes of several Salmonella enterica serovars and of E. coli ED1a (NC 011754). The iol genes, however, are absent in Salmonella enterica serovars such as Typhi, Parathypi A and Choleraesuis, indicating that the utilization of myo-inositol is not a common ability of Salmonella strains and does not play a role during systemic infection. An intriguing property of Salmonella enterica sv. Typhimurium growth on myo-inositol is the long lag phase of ~2 days, which is, however, reduced strongly upon the deletion of the repressor IolR or the addition of bicarbonate to simulate conditions in the intestine (Kröger et al., 2011). Recent in vivo screening approaches indicate that iol genes play a role in Salmonella enterica sv. Typhimurium virulence in mice, pigs, chicken and calves (Carnell et al., 2007; Chaudhuri et al., 2009, 2013; Lawley et al., 2006). The srfJ gene, also located on GEI4417/4436, encodes a putative glycosyl ceramidase or glycohydrolase. It is induced within host cells by the TCS SsrAB, which controls the expression of SPI2 genes, and negatively regulated by the repressor IolR (Cordero-Alba et al., 2012; Worley et al., 2000). Deletion of srfJ (STM4426) results in a slight attenuation with respect to the systemic virulence in mice (Ruiz-Albert et al., 2002). Moreover, a yet unknown regulatory gene was shown to be upregulated in the gut using an enteritis mouse model (Rollenhagen & Bumann, 2006). Several gut pathogens carry iol gene homologues (Table 1), although a contribution of myo-inositol degradation to the virulence properties of Clostridium perfringens or Y. enterocolitica has not been demonstrated. Interestingly, the iol gene cluster is upregulated in Enterococcus faecalis during replication in monoassociated mice (Lindenstrauß, 2012).

Parallels to nutrient degradation and metabolite availability in food

An infection by foodborne pathogens starts with contamination of food in which the bacteria possibly proliferate before they are orally taken up by a mammalian host. In light of the findings reviewed above, it might be speculated that there are analogies between the food and the gut environments with respect to nutrient availability and the interdependencies or competition between pathogenic and non-pathogenic microbes. Indeed, most foods are considered nutrient-rich and provide partial anaerobic conditions. Muscle tissues of meat animals comprise 18% protein and 3% fat, and are a highly favourable growth medium for bacteria with readily available glycogen, peptides and amino acids, as well as metal ions and soluble phosphorus (Jay, 2000). Fruit and vegetable products are characterized by a high mean content of 8.6% carbohydrates (polysaccharides, oligosaccharides, monosaccharides including l-rhamnose, l-fucose and gluconate), but contain only 1.9% proteins and 0.3% fat. On the one hand, there is a strong competition with spoilage bacteria, moulds and yeasts for nutrients, at least in raw meat or milk and on plants, eventually resulting in a nutrient-exhausted environment similar to that encountered in the gut. For example, glucose is known to be depleted in meat when bacterial populations exceed 10⁹ c.f.u. cm⁻² (Jackson et al., 1997). On the other hand, foodborne pathogens also profit from the disintegrative enzymic activities of (non-pathogenic) spoilage micro-organisms. In milk, bacteria unable to utilize lactose depend on the lipolytic and proteolytic activities provided by other microbes that make milk fat available that is enclosed by the globule membrane. Plant-contaminating pathogens might take advantage of microbes producing enzymes such as pectinases and cellulases that degrade polysaccharides (Jay, 2000). Spoilage of freshly slaughtered meat occurs mainly by bacteria with lipolytic and proteolytic activities (Clostridia, Pseudomonas and members of the Enterobacteriaceae) and yeast. Proteolysis occurs at populations >10⁸ c.f.u. cm⁻², when spoilage is well advanced and bacteria are approaching their maximal cell density (Dainty et al., 1975). Lipases catalyse
the hydrolysis of phospholipids as an abundant component of the tissue membranes to glycerol and free fatty acids. In the case of phosphatidylethanolamine, ubiquitously present in food, this activity results in the release of ethanolamine. We recently chose utilization of this metabolite for the hypothesis that this metabolic adaptation required for full virulence properties of *Salmonella enterica* sv. Typhimurium also contributes to its proliferation in food. It was observed that growth of a mutant that lacks EutR, the inducer of the eut and cob/cbi genes, was reduced significantly in milk and in egg yolk (Srikumar & Fuchs, 2011). The eutS gene of *Salmonella enterica* sv. Typhimurium was shown to be upregulated strongly in pasteurized milk as well as in egg yolk and an eutR deletion mutant was significantly growth attenuated with both these substrates (Srikumar & Fuchs, 2011).

In the case of processed and fermented products, foodborne pathogens might contaminate a largely non-colonized and thus non-competitive milieu, but lack the help of spoilage bacteria to degrade otherwise non-accessible food macromolecules. Processed food is characterized by a mixture of anaerobic as well as aerobic microenvironments. Moreover, food processing and preservation methods, such as cooling, fermentation, heating, smoking or drying, often result in the lowering of pH or water activity, formation of a solid matrix, or carbohydrate depletion and thus in less favourable growth conditions for enteropathogens. In comparison with fluid milk, dairy products provide substantially different growth conditions that are less supportive for micro-organisms. Thus, foodborne pathogens growing on or in processed food have developed strategies to overcome those limitations, especially by broadening their temperature range for growth, and, as addressed here, by developing common and specific metabolic capacities.

**Conclusions**

Metabolic versatility under nutrient constraints is a prerequisite for pathogens that survive and proliferate successfully in different niches and compartments during their life cycle. The metabolites mentioned in the section ‘A
frugal or an opulent meal?’ are readily available in the gut upon the activity of the commensal microbiota (Fig. 3). Their usage by enteropathogens only requires uptake systems and a standard repertoire of metabolic enzymes, and might help to overcome auxotrophies or bottlenecks in anabolic pathways. In contrast, the complex and more unusual degradation properties of enteric pathogens described in the further sections require larger gene sets that are encoded by distinct chromosomal regions. These constitute specific metabolic adaptations not common to members of the gut microbiota, and provide energy, carbon or nitrogen to pathogens under competitive circumstances and thus in vivo growth advantages over bacteria lacking this function. Due to their distinct G/C content, the genomic fragments responsible for the utilization of myo-inositol, ethanolamine, propanedioic and sialic acids might be termed metabolic islands (Almagro-Moreno & Boyd, 2009a; Dobrindt et al., 2004). Similar to pathogenicity islands contributing to virulence, it is assumed that these genetic determinants encoding metabolic functions have been acquired by horizontal gene transfer to expand the metabolite target spectrum of the enteropathogens (Rohmer et al., 2011).

Depending on the diet, the microbiota and the host response, the carbon and energy sources mentioned above are available in varying amounts in the gut. Further research is required to delineate if, and to what extent, their utilization indeed contributes to proliferation of pathogens during infection. Due to their high metabolic flexibility, the enteric pathogens can be assumed to be well adapted under disturbances of the gut metabolome and nutrient composition, content of oxygen, competition for nutrients in the gut. Depending on the diet, the microbiota and the host response, the carbon and energy sources mentioned above are available in varying amounts in the gut. Further research is required to delineate if, and to what extent, their utilization indeed contributes to proliferation of pathogens during infection. Due to their high metabolic flexibility, the enteric pathogens can be assumed to be well adapted under disturbances of the gut metabolome and nutrient composition, content of oxygen, competition for nutrients in the gut.

As delineated above, foodborne enteropathogens encounter comparable challenges in food and in the gut with respect to nutrient composition, content of oxygen, competition with dominating microbiota and dependency on other microbes to degrade macromolecules. We therefore conclude that the ability of enteric pathogens to utilize substrates such as inositol, ethanolamine, fucose or sialic acid provides a permanent selection advantage during proliferation in food and, following ingestion, during subsequent stages of infection, i.e. from food to cell.

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