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Mini Review

Sugar metabolism, an additional virulence factor in enterobacteria

Chantal Le Bouguénec[a,b,*], Catherine Schouler[c]

[a] Institut Pasteur, Unité de Biologie des Bactéries Pathogènes à Gram-Positif, Département de Microbiologie, 28 rue du Dr Roux, F-75015 Paris, France
[b] CNRS, URA2172, F-75015 Paris, France
[c] INRA, UR1282, Infectiologie Animale et Santé Publique, Centre de Recherche de Tours, F-37380 Nouzilly, France

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ABSTRACT

Enterobacteria display a high level of flexibility in their fermentative metabolism. Biotyping assays have thus been developed to discriminate between clinical isolates. Each biotype uses one or more sugars more efficiently than the others. Recent studies show links between sugar metabolism and virulence in enterobacteria. In particular, mechanisms of carbohydrate utilization differ substantially between pathogenic and commensal *E. coli* strains. We are now starting to gain insight into the importance of this variability in metabolic function. Studies using various animal models of intestinal colonization showed that the presence of the *fos* and *deoK* loci involved in the metabolism of short-chain fructoligosaccharides and deoxyribose, respectively, help avian and human pathogenic *E. coli* to outcompete with the normal flora and colonize the intestine. Both PTS and non-PTS sugar transporters have been found to modulate virulence of extraintestinal pathogenic *E. coli* strains. The *vpe*, GimA, and *aec35-37* loci contribute to bacterial virulence in vivo during experimental septicemia and urinary tract infection, meningitis, and colibacillosis, respectively. However, in most cases, the sugars metabolized, and the precise role of their utilization in the expression of bacterial virulence is still unknown. The massive development of powerful analytical methods over recent years will allow establishing the knowledge of the metabolic basis of bacterial pathogenesis that appears to be the next challenge in the field of infectious diseases.

Introduction

The bacterial family *Enterobacteriaceae* includes many well-studied bacteria that are normal inhabitants of the intestine of healthy individuals. Other members of this family are found in soil, plants, and water. However, under certain circumstances, some enterobacteria, within genera such as *Escherichia*, *Salmonella*, and *Yersinia*, can also be dangerous pathogens in humans and animals. Studies over the last 30 years have identified and described numerous virulence factors (VFs) – including adhesins/invasins, toxins, iron acquisition systems, and mechanisms to evade the immune response – and the genes encoding them. There is now a substantial body of knowledge about the development of intestinal and extraintestinal syndromes caused by these bacteria. However, the characterization of these VFs is not sufficient to understand infectious disease mechanisms. Indeed, we also need to understand how pathogenic bacteria adapt their metabolism to derive carbon and energy from the environment, allowing them to grow, to survive, and to colonize their hosts at intestinal and extraintestinal sites. Studies of the links between metabolism and virulence could provide not only new insights into host–pathogen interactions, but also new perspectives in understanding the metabolic basis of bacterial pathogenesis.

A number of physiological studies have shown that the regulation of expression of many VFs is controlled by nutrient availability (Somerville and Proctor, 2009). However, the regulatory mechanisms linking a particular metabolic system to the production of a specific VF are still poorly understood. A recent symposium brought researchers in these different fields together for the first time (Metabolism Meets Virulence, International Symposium, Hohenkammer, Germany, April 2009). Pathogenic enterobacteria have developed two strategies to compete with normal flora and colonize infectious sites. They can modify their metabolism in response to environmental changes by regulating catabolic pathways, the components of which are encoded by the core genome. Many transcriptomic, proteomic, and metabolomic studies have been carried out to decipher the essential pathways in bacteria during infection. Indeed, comparative genomic and growth analyses of the pathogenic *E. coli* O157:H7 (strain EDL933) and the non-pathogenic *E. coli* K-12 (strain MG1655) have shown that there are no major differences in the gene systems that encode and regulate the pathways for carbon source utilization and no differences in nutritional usage in vitro between these two strains.

* Corresponding author at: Institut Pasteur, Unité de Biologie des Bactéries Pathogènes à Gram-Positif, Département de Microbiologie, 28 rue du Dr Roux, F-75015 Paris, France. Tel.: +33 1 40613280; fax: +33 1 40613640.
E-mail addresses: chantal.le-bouguenec@pasteur.fr, clb@pasteur.fr (C. Le Bouguénec).

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However, these strains use different carbon sources for the colonization of the streptomycin-treated mouse intestine (Fabich et al., 2008). Pathogenic bacteria can also adapt to changing nutrient supplies by expressing specific genes that are not present in the genomes of commensal isolates. For example, uropathogenic bacteria need to take advantage of available nutrients present in urine, comprising a dilute mixture of amino acids and small peptides. One potential nutrient in urine is D-serine, which is toxic for commensal strains but is degraded by a D-serine deaminase (DsdA) in uropathogenic strains. Approximately 85% of pyelonephritis- and urosepsis-associated *E. coli* isolates carry at least one *dsdXA* operon for D-serine utilization. Recently, a *dsdA* gene was identified in uropathogenic *Staphylococcus saprophyticus* isolates, but not in other staphylococcal species. One study showed a uropathogenic *E. coli* CFT073 *dsdA* mutant displaying a hyper-colonization phenotype in a murine model of urinary tract infection (UTI) (Anfora et al., 2007); however, another study showed a *S. saprophyticus dsdA* mutant to be less virulent than the parental strain in experimental infections (Sakellaris et al., 1999). Thus, catabolism of D-serine may act as either a fitness trait or a signal for virulence gene expression, depending on the strain and environmental conditions.

Carbohydrate metabolism is important for virulence mechanisms in enterobacteria

The metabolic flexibility of enterobacteria is related to genetic diversity and dynamic organization of the genome, which seem to be general characteristics of these bacteria. The sequencing of more than 50 genomes of *E. coli* strains has been completed or is in progress (http://www.genomesonline.org/gold.cgi). This has revealed differences encompassing up to 1 Mb of sequence in genomes ranging from 4.5 to 5.5 Mb in size. Although comparative and functional analyses of the genome have improved our fundamental understanding of the virulence mechanisms in pathogenic strains (Iguchi et al., 2009; Rasko et al., 2008), we have also gained insight into the modes of adaptation of commensal and pathogenic *E. coli* isolates to different ecological niches, in particular the important role of carbohydrate metabolism in these processes. Subtractive hybridization experiments identified a sucrose-specific phosphotransferase system found only in extraintestinal virulent *E. coli* isolates (Sorsa et al., 2007). DNA microarray analysis of 19 commensal *E. coli* isolates from the intestinal microbiota of humans and animals showed that the functions of a non-negligible proportion of hyperdivergent genes are related to carbohydrate transport and metabolism (Grasselli et al., 2008). Analysis of the complete genome of the *E. coli* commensal strain SE11 led to the identification of strain-specific genes linked to oligosaccharide metabolism (Oshima et al., 2008). More recently, comparison studies between *Shigella* genomes and the complete genome of 20 commensal and pathogenic *E. coli* strains suggested an important adaptive role for metabolic diversification in virulence, highlighting the roles of carbohydrate metabolism and transport (Touchon et al., 2009).

Carbohydrate transporters in enterobacteria

Dozens of families of primary and secondary transporters allowing the uptake of essential nutrients have been described (Saier, 2000). Two families have been found to appear ubiquitously in all classifications of living organisms. There are the ATP-binding cassette (ABC) superfamily and the major facilitator superfamily (MSF). Functionally and structurally different sugar transporters have been identified in enterobacteria. Primary, active ABC transporters, coupling transport against a concentration gradient to ATP hydrolysis, mediate the uptake of sugars such as maltose. Various MFS permeases belonging to different families have been characterized, the best-characterized of these being the LacY permease, which belongs to the oligosaccharide:H^+ symporter family (Saier et al., 1999). Another family of transporters, unique to prokaryotes, catalyzes the uptake of carbohydrates, particularly hexoses, hexitols, and disaccharides. These phosphoenolpyruvate (PEP):carbohydrate phosphotransferase system (PTS) transporters allow substrates, having crossed the outer membrane by facilitated diffusion through porins, to cross the inner membrane using PEP as an energy source. The PTS system consists of two general components, both of which are cytoplasmic energy-coupling proteins: enzyme I (EI) and histidine phosphocarrier protein (HPr), and various membrane-associated sugar permeases/phosphotransferases [enzymes II (EII)]. Each EII complex consists of one or two hydrophobic integral membrane domains (domains C and D) and two hydrophilic domains (domains A and B). EII complexes may exist as distinct proteins or as single multi-domain proteins. This system is a sensory and transport system at the same time. In a sequence of four reactions, a phosphoryl group is transferred from PEP to the incoming sugar. Initial autophosphorylation of EI, using PEP as a substrate, is followed by transfer of the phosphoryl group from EI to HPr. EIIA catalyzes the self-phosphoryl transfer from HPr, allowing the phosphoryl group to be transferred to histidine or cysteine residues of EIIB. EIIC and EIID are integral inner-membrane proteins that catalyze the transport step of their substrate. This substrate is phosphorylated by the appropriate sugar-specific EIIB. Recent studies have shown links between carbohydrate transporters belonging to these different categories and the virulence of pathogenic enterobacteria, particularly of pathogenic *E. coli* strains in various animal models (see below). Several MSF transporters and EII complexes, identified specifically in the genomes of intestinal and extraintestinal pathogenic *E. coli*, have been demonstrated to be linked to the colonization of the host at various sites. We discuss these transporters and complexes in this review. An ABC transporter-encoding sequence was recently found to be associated with *E. coli* isolates belonging to the B2 phylogenetic group, which comprises most of the extraintestinal virulent isolates (Le Bouguénec et al., unpublished data).

Carbohydrate metabolism and colonization of the intestine by commensal and pathogenic enterobacteria

The intestine is the primary niche of most enterobacteria among which *E. coli* is predominant (Harmsen et al., 2002). Colonization of the intestine is the first step of host infectivity for pathogenic enterobacteria, including all categories of pathogenic *E. coli*. Intestinal pathogenic *E. coli* strains are rarely found in the fecal flora of healthy hosts. Extraintestinal pathogenic *E. coli*, however, stably colonize the intestine without inducing clinical symptoms. These bacteria constitute the predominant *E. coli* in approximately 20% of healthy people (Johnson and Russo, 2002). Intestinal populations of bacteria control each other by metabolic competition for limiting nutrients metabolized under the physiological conditions of the gut (Freter et al., 1983). To become established in the intestine, foreign *E. coli* strains (in particular, pathogenic isolates) must either compete for nutrients with resident *E. coli* strains or metabolize different nutrients. Thus, the in vivo metabolic requirements of pathogenic and commensal strains may differ. Carbohydrate metabolism is widely accepted to be the nutritional basis for colonization of the intestine by *E. coli* and maintenance of the strains (Chang et al., 2004). Thus, given the importance of the genes encoding these pathways in the diversity of the species, a number of studies have focused on the role of sugar metabolism in the colonization of the intestine (Table 1 and see below).

Mammals are well equipped to absorb simple sugars such as glucose and galactose in the proximal portion of their small intestine.