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Comparative genomics of transport proteins in probiotic and pathogenic *Escherichia coli* and *Salmonella enterica* strains

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Abstract

Escherichia coli is a genetically diverse species that can be pathogenic, probiotic, commensal, or a harmless laboratory strain. Pathogenic strains of *E. coli* cause urinary tract infections, diarrhea, hemorrhagic colitis, and pyelonephritis, while the two known probiotic *E. coli* strains combat inflammatory bowel disease and play a role in immunomodulation. *Salmonella enterica*, a close relative of *E. coli*, includes two important pathogenic serovars, Typhi and Typhimurium, causing typhoid fever and enterocolitis in humans, respectively, with the latter strain also causing a lethal typhoid fever-like disease in mice. In this study, we identify the transport systems and their substrates within seven *E. coli* strains: two probiotic strains, two extracellular pathogens, two intracellular pathogens, and K-12, as well as the two intracellular pathogenic *S. enterica* strains noted above. Transport systems characteristic of each probiotic or pathogenic species were thus identified, and the tabulated results obtained with all of these strains were compared. We found that the probiotic and pathogenic strains generally contain more iron-siderophore and sugar transporters than *E. coli* K-12. Pathogens have increased numbers of pore-forming toxins, protein secretion systems, decarboxylation-driven Na^+ exporters, electron flow-driven monovalent cation exporters, and putative transporters of unknown function compared to the probiotic strains. Both pathogens and probiotic strains encode metabolite transporters that reflect their intracellular versus extracellular environments. The results indicate that the probiotic strains live extracellularly. It seems that relatively few virulence factors can convert a beneficial or commensal microorganism into a pathogen. Taken together, the results reveal the distinguishing features of these strains and provide a starting point for future engineering of beneficial enteric bacteria.

Keywords

probiotics; pathogens; *E. coli*; *Salmonella enterica*; transport proteins; physiology

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1. Introduction

Salmonella enterica and *Escherichia coli* are two closely related enteric bacteria that cause a plethora of human and animal diseases [1-5]. In spite of their close ancestry, they are the causative agents of very different types of diseases [6]. The better-characterized *E. coli* also includes probiotic and commensal strains that, in contrast to the pathogens, benefit or have little effect on the host [7, 8]. In view of the diverse *in vivo* attributes of these bacteria, we decided to examine their transport systems to determine if distinguishing features, responsible for their diverse host-microbe interactions could be identified.

Probiotic bacteria are defined as live microorganisms that, when administered orally in adequate quantities can colonize the intestines and confer upon the host health benefits, and prebiotic substances are defined as nutrients that stimulate the growth of beneficial species in the body. Adaptive co-evolution of humans and bacteria over millennia has resulted in symbiotic relationships in which both partners benefit [9-11]. The mechanisms of action of probiotics have been generalized to (i) strengthening the intestinal barrier, (ii) modulating the immune response, (iii) secreting antimicrobial compounds, and (iv) competing with pathogens for mucosal binding sites [7]. However, some mechanisms are strain-specific or are poorly defined [12].

E. coli is the leading cause of preventable infant mortality worldwide, and it causes several pathological conditions. However, it also includes commensal and probiotic strains [13]. *E. coli* genomes are composed of two gene pools: the core and the variable portions. The core pool is conserved between strains, while the variable pool is strain-specific [7]. *E. coli* Nissle 1917 is a well-studied probiotic strain that has been shown to exhibit anti-invasive effects on bacterial pathogens without requiring physical contact with the invasive bacteria or the epithelial cells [14, 15]. However, the mechanism of action of the closely related probiotic *E. coli* O83 is less well understood, and it is thought to directly compete with bacterial pathogens through adhesion, iron acquisition, and bacteriocin secretion [15]. Regardless, both strains have been shown to be clinically useful in ameliorating diseases, such as inflammatory bowel disease [8].

Infections due to *Salmonella* remain a major health concern, resulting in about 160,000 deaths and 94 million illnesses annually [16]. In contrast to *E. coli*, there are no known probiotic *Salmonella* species, but *S. enterica* includes several intracellular pathogens. *S. enterica* is divided into six subspecies based on flagellar structure and the presence of certain antigens; it contains over 2500 serotypes [3]. In this study, we focus on two medically relevant serotypes of *S. enterica*, *S.* Typhi and *S.* Typhimurium, that cause typhoid fever and enterocolitis in humans, respectively, although the latter also causes a typhoid fever-like condition in mice [3-5].

In a previous study, Tang and Saier reported that pathogenic strains of *E. coli* possess complements of transport proteins that provide clues as to the systems that impart virulence [17]. Many transporters were unique to certain pathogens, and their presence or absence explained, in part, the virulence properties. For example, sets of protein secretion systems for export to the cell surface or injection of effector proteins into host cells were generally

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strain-specific. Pathogens also have increased numbers of iron siderophore receptors and ABC iron uptake transporters compared to *E. coli* K-12, but the numbers and types of low-affinity secondary iron carriers were uniform in all strains. Each pathovar encodes a different set of pore-forming toxins and virulence-related outer membrane proteins lacking in the non-virulent K-12 strain [17].

The goal of this study was to extend these results to probiotic *E. coli* and key pathogenic *Salmonella* strains for comparative purposes. Seven *E. coli* strains, two extracellular pathogens (strains UMN026 and O157:H7), two intracellular pathogens (strains ABU83792 and CFT073), two probiotic lines (O83 and Nissle 1917), and K-12 as well as two intracellular *Salmonella* pathogens (*S. enterica* strains CT18 (Typhi) and LT2 (Typhimurium)), were examined to analyze the distributions of transport systems and their substrates in order to elucidate differences between substrates transported and transporter types that might confer probiotic versus pathogenic character. Table 1 presents the basic traits of these nine strains.

2. Materials and Methods

2.1 Genome-BLAST (G-BLAST) search for transport proteins

The proteomes of seven *E. coli* strains (K-12, O83, Nissle, O157, UMN, CFT, and ABU) and two *S. enterica* strains (CT18 and LT2) were obtained from GenBank based on the draft quality and completeness of their sequenced genomes, as well as their relevance to human health. They were then screened against the Transporter Classification Database (TCDB; www.tcdb.org) for transport protein homologues in October, 2016 using G-BLAST [18]. G-BLAST is especially designed for this purpose, as it uses FASTA-formatted protein sequences from the genome as queries to search for homologous transport proteins within TCDB and then retrieves information from the top TC hit (TCID number, number of amino acyl residues (aas), predicted numbers of transmembrane segments (TMSs), TMS overlap between query and hit, and e-value), indicating the degree of similarity between the query and hit proteins [19]. To predict the numbers of TMSs, G-BLAST uses the Web-based Hydropathy, Amphipathicity, and Topology (WHAT) program, which aligns the plots of hydrophobicity and amphipathicity through the length of the protein [20, 21]. The WHAT program was used to display hydropathy and amphipathicity profiles of individual proteins with a window size of 19 amino acids and a viewing angle of 100° for α-helices or 180° for β-strands in order to compare the topologies of the query proteins with their top TC hits [21]. Proteins with no TMSs were not automatically excluded since many multicomponent systems contain soluble components that might be potential transport protein homologues.

2.2 Examination of transport protein homologues

An arbitrary e-value cutoff of 0.0001 was initially used following G-BLAST searches. The remaining proteins that had e-values of 0.0001 or more were manually examined using topological data to determine if they were likely to be true homologues of recognized transport proteins, or if they were false positives. Since two proteins showing homology in hydrophilic regions can give small e-values, manual examination was required in order to avoid including well-scoring proteins that were not actually homologous in the

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transmembrane domains. By using the hydropathy profile generated by WHAT, it could be determined whether the program had missed a TMS or predicted a TMS in an incorrect region. This also allowed for the inclusion or exclusion of TC hits based on numbers of TMSs, as well as TMS locations.

Proteins with moderate e-values, between 0.0001 and e^{-8}, represent a range in which there could be distant transport protein homologues, and thus, they were examined in closer detail using the aforementioned steps. Low-scoring hits that proved to be recognizable transport proteins were incorporated into TCDB.

2.3 Identification of substrates transported

Authentic transport protein homologues were assigned substrates according to TCDB hit entries. For entries of unknown function, the genome context of genes encoded within operons or information obtained from the scientific literature was used to deduce their functions.

3. Results

3.1 Overview of transporter types

Following the conventions of TCDB, transporters are organized into five well-defined categories, classes one to five, and two less well-defined categories, classes eight and nine. The five well-defined classes are (1) channels (2) secondary carriers (3) primary active transporters, (4) group translocators, and (5) transmembrane electron flow carriers. Meanwhile, the latter two classes include (8) auxiliary transport proteins and (9) transporters or putative transporters of unknown function or mechanism of transport [22, 23].

To analyze the distribution of transport proteins within each of the seven *E. coli* and two *Salmonella* strains, their proteomes were screened against TCDB using G-BLAST. The complete results are shown in detail in Table S1, while Table 2 summarizes the distribution of the subclasses of transporters found in each of the seven *E. coli* strains and two *Salmonella* strains according to TC number. Surprisingly, the probiotic strains and *E. coli* K-12 contain fewer transport proteins than their pathogenic counterparts (855, 873, and 842 versus 886, 874, 910 and 898 respectively). *Salmonella* CT18 contains the fewest transport proteins, 821, while *Salmonella* LT2 has 862. In general, all nine strains show similar distributions of the different transporter classes. The most obvious differences are among pore-forming toxins, auxiliary transport proteins, and putative transport proteins in TC subclass 9.B.

TC subclass 1.A represents α-type channels except for holins, which are found in subclass 1.E [24]. The probiotic and pathogenic strains of both *E. coli* and *Salmonella* show a similar distribution of TC subclass 1.A, each having 33-35 such proteins. However, *E. coli* ABU contains the most such channels, 38. Similar to our previous study [17], *E. coli* K-12 contains fewer (29) such systems.

TC subclass 1.B includes β-barrel porins that are located in the outer membranes of these Gram-negative bacteria [25]. Similar to the distribution of TC subclass 1.A, the probiotic

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and pathogenic strains of *E. coli* contain more of these types of proteins than *E. coli* K-12 and the *Salmonella* strains. *E. coli* K-12 contains 64 such proteins, and both *Salmonella* CT18 and LT2 contain 62 proteins, whereas the remaining four strains possess 74-86.

TC subclass 1.C includes pore-forming toxins [24]. Three of the *E. coli* pathogenic strains, CFT073, ABU, and O157, each contain 10-12 such proteins. Surprisingly, both *Salmonella* CT18 and LT2 contain fewer pore-forming toxins than the *E. coli* pathogens, with only eight. The probiotic strains, O83 and Nissle, contain only two and four such toxins, respectively, while *E. coli* K-12 contains six.

TC subclass 1.E consists of holins. The nine strains show consistent patterns within this subclass. *E. coli* Nissle and CFT073 each encodes seven holins. The remaining *E. coli* strains have nine to eleven. The two *Salmonella* strains, CT18 and LT2, contain on average more holins than the seven *E. coli* strains with 11 and 13, respectively.

Secondary carriers represent the largest groups of transport systems in all nine strains with approximately 30% of the transport proteins in each strain falling within this class. However, these proteins and primary active transport proteins are found in similar number. Within TC subclass 2.A, each strain contains 225-280 porters. However, the two *Salmonella* strains fall in the lower part of this range, with CT18 containing 225 proteins and LT2 containing 254. The remaining class 2 proteins fall into TC subclass 2.C, ion-gradient-driven energizers of motility and outer membrane transport; all strains have a similar pattern, having a range of 3 to 5 such proteins.

The second largest number of transport protein types in all five strains is relegated to primary active transporters that comprise 26-30% of all transport proteins in these strains. However, the number of such systems is far fewer than the number of secondary carriers because the former are usually multicomponent systems while the latter are usually single component systems. TC subclass 3.A includes phosphate bond-hydrolysis-driven transporters. *E. coli* K-12 contains the fewest of these proteins, numbering only 226. The remaining eight strains contain between 243-270. TC subclass 3.B includes decarboxylation-driven transporters. These transporters are absent in the probiotic strains and ABU, while the remaining *E. coli* strains contain two each; the two *Salmonella* strains both contain six such proteins. TC subclass 3.D includes oxidoreduction-driven transporters. The probiotic *E. coli* and *Salmonella* strains contain 36-39 of these proteins, while the remaining *E. coli* strains each contains between 36-50.

TC subclass 4.A consists of phosphotransfer-driven sugar transporting group translocators. On average, the *Salmonella* strains contain fewer such proteins, 34-42, compared to the *E.* coli strains, which contain 47-55 of these proteins, except for *E. coli* K-12, which contains 43. TC subclass 4.B includes nicotinamide ribonucleoside uptake transporters and their homologues, which number two or three across all nine strains. TC subclass 4.C includes acyl-CoA ligase-coupled transporters, which number two or three across all nine strains. TC subclass 4.D includes polysaccharide synthase/exporters (glycosyl transferases). All *E. coli* strains contain three or four of these proteins, while CT18 and LT2 have six such proteins each.

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TC subclass 5.A includes transmembrane two-electron carriers. There are 26-30 of these proteins in each of the nine strains. TC subclass 5.B includes transmembrane one-electron carriers. While these are absent in the probiotic strains, the remaining seven strains have two to five of these carriers.

TC subclass 8.A includes auxiliary transport proteins that do not participate directly in the transport process, but facilitate this process. The probiotic *E. coli* and *Salmonella* strains contain 15-17 such proteins, while the rest contain between 21-23.

The remainder of the proteins in each strain falls into TC subclasses 9.A, known transporters that function by unknown mechanisms of action, or 9.B, putative transporters where the evidence for a transport function is insufficient to establish such a function. Each of the nine strains shows 7-12 proteins from TC subclass 9.A, but in TC subclass 9.B, the probiotic strains have 47 and 52 proteins, while all the pathogenic strains and *E. coli* K-12 have 62-66.

3.2 Differences in transported substrates between probiotic and pathogenic strains

To better understand the contribution of transport systems to probiotic or pathogenic character, the probable substrate specificities of most transport systems were predicted. While the detailed results are tabulated in Table S1, Table 3 provides an overview of predicted substrate types transported, and it can be seen that the probiotic strains, *E. coli* K-12, and the *Salmonella* strains generally contain fewer transport proteins than the pathogenic strains (804-858 versus 852-887). The distribution of transported substrates is similar across all nine strains, though there are notable differences.

The results in Table 3 show that the probiotic *E. coli* strains contain fewer transporters of unknown function (97-105 versus 113-128) than the pathogens and *E. coli* K-12. Similarly, the pathogenic strains contain more protein and peptide transport proteins on average than the probiotic strains or *E. coli* K-12 (126-159 versus 129-131). Many are probably for secretion of virulence factors. With regards to siderophore transport, both *Salmonella* strains have the lowest numbers of transporters, 16 and 19, followed by *E. coli* K-12 with 24. Both probiotic and pathogenic *E. coli* strains have higher numbers of iron-siderophore transporters (26-38 versus 16-24). Interestingly, *E. coli* O157 contains fewer polysaccharide transporters than all the other strains (18 versus 21-26).

3.3 Metabolites transported by extracellular versus intracellular strains

Several metabolite porters were uniquely identified in either the extracellular or the intracellular pathogens; the results are summarized in Table 4. It can be seen that the *Salmonella*, *E. coli* CFT, and ABU strains that reside intracellularly within the host, contain more transporters that are specific to metabolites that reside within the cytoplasm (i.e., di- and tricarboxylates, phosphoglycerate, phosphoenolpyruvate, 2-keto-3-deoxygluconate, glucose, fructose etc.). These substrates are often glycolytic and Krebs cycle intermediates. In contrast, strains that reside extracellularly, the two probiotic strains, *E. coli* O157, UMN, and K-12, contain more transporters that are specific for nutrients that are normally found in the extracellular millieu (i.e., raffinose, melibiose, cellobiose, maltose, etc.). This trend had been noticed previously when just pathogens were analyzed [17].

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3.4 Some, but not other types of transporters that contribute to pathogenesis are also found in probiotic strains

Protein secretion systems, iron transporters, and toxins were examined because of their known involvement in pathogenicity. The results are summarized in Tables 5, 6, and 7, respectively. As presented in Table 5, the occurrence of protein secretion systems is disparate in the different strains with no obvious pattern. Homologues of components of type III secretion systems (T3SSs) are present in all strains, though only *E. coli* O157 and the two *Salmonella* strains have two T3SSs each. As reported in our previous studies, the presence of type VI secretion system (T6SS) constituents is common to pathogenic strains, but these proteins are also present in the probiotic strains [17]. Nevertheless, the pathogenic strains contain more of these proteins than the probiotic strains (Table 5). This suggests that while the former may be complete, the latter are not. As expected, common to all nine strains is the presence of the general secretory pathway and the flagellar protein export system (TC#s 3.A.5.1.1 and 3.A.6.2.1, respectively).

With regards to iron transporters, the pathogenic strains share with the probiotic strains most of their secondary iron transport systems and some primary active transporters as well (Table 6). However, the probiotic strains have slightly more iron-siderophore uptake systems than the pathogens, and far more than both *Salmonella* strains. It appears that three of the iron transport systems are unique to the probiotic strains and may catalyze the uptake of Fe^{3+}-ferrichrome (TC# 1.B.14.1.15), heme (TC# 1.B.14.10.1), and Fe^{3+}-vibrioferrin (TC# 3.A.1.14.8). However, both probiotic and pathogenic strains contain more high-affinity than low-affinity iron transporters, consistent with previous results (Table 6) [17].

Unlike the other two categories of transport systems, toxins are virtually absent from the probiotic strains except for colicin V (TC# 1.C.31.1.3) and cytotoxic fimbrial subunit transporters (TC#s 1.C.80.1.2 and 3) as shown in Table 7. In accordance with the presence of T3SSs, members of the type III-target cell pore (IIITCP) family are only found in *E. coli* O157 and the *Salmonella* strains. Consistent with our previous results, various hemolysins, Shiga toxins, clostridial cytotoxins, and *Serratia*-type pore-forming toxins were all identified in pathogenic strains, though several are absent from both *Salmonella* strains; these were discussed previously [17].

3.5 Transporters found exclusively in either *S.* Typhi (CT18) or *S.* Typhimurium (LT2)

To better understand the differences between the two strains of *Salmonella*, their exclusive transporters were examined, and the results are summarized in Table 8. CT18 contains five transporters that are not found in any of the *E. coli* strains or LT2, most of which are ABC transporters (TC subclass 3.A). In contrast, LT2 contains 14 exclusive transporters; almost all of which are secondary carriers (TC subclass 2.A). The presence of more secondary carriers in LT2, but more ABC transporters in CT18 suggests that Typhi lives in a more anaerobic environment than Typhimurium (see Discussion).

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4. Discussion

Probiogenomics, the sequencing and analysis of probiotic and commensal gut bacteria, is a powerful tool that can facilitate the comparative analyses of large numbers of proteins to reveal similarities and differences between different species or strains of organisms [7]. The genomes of seven *E. coli* strains (two probiotic, two extracellular pathogens, two intracellular pathogens, and *E. coli* K-12) as well as two *Salmonella* pathogens were screened against TCDB to identify homologues of established or putative transport proteins. This allowed us to obtain nearly complete sets of transport systems, based on current knowledge of these systems. The sum total of the proteins comprises the organisms' "transportosomes" [26]. These transportomes were then used to compare and contrast the presence or absence of transport systems, as well as the substrates transported, in each of the nine strains.

An interesting observation resulting from the analyses described in this paper confirmed and extended the results of Tang and Saier concerning transport systems found in intracellular versus extracellular pathogens [17]. Probiotic bacteria display transport systems characteristic of extracellular organisms. Thus, extracellular bacteria have transporters specific for a variety of disaccharides, glycosides, and monosaccharides seldom found inside animal cells, as well as osmolytes, and certain vitamins and vitamin precursors. It is also interesting that sugar transporters found in the probiotics often differ from those found in the pathogenic strains (Table 4). By contrast, the intracellular pathogens exhibit many transporters not found in the extracellular strains, and a majority of these are present in the two *S. enterica* strains, known to live primarily in the host cell cytoplasm. These systems are usually specific for intracellular metabolites, such as intermediates of glycolysis and the Krebs cycle, as well as other intracellular metabolites and certain drugs. It seems likely that many of the genes that distinguish intracellular from extracellular enteric bacteria were obtained relatively recently during their evolutionary histories by horizontal gene transfer.

It has been shown that many ingested bacteria can ferment carbohydrates non-digestible by humans, converting them into short-chain fatty acids such as acetate, lactate, proprionate, and butyrate [27, 28]. While acetate and propionate are used by the liver for lipogenesis and gluconeogenesis, butyrate is metabolized in the colonic epithelium and may function as a histone deacetylase inhibitor, thereby regulating mammalian transcription [28]. These short-chain fatty acids have also been shown to signal, by binding to G-protein-coupled receptors, for different functions, depending on the cell type. This effect can range from suppression of inflammation by neutrophils to improvement of insulin secretion by enteroendocrine L-cells [28]. Therefore, *E. coli* Nissle and O83 may antagonize gut pathogens by affecting the host immune system, in part by virtue of their unique sugar metabolic capabilities.

In both *E. coli* and *Salmonella*, iron is required to sustain cellular respiration and is crucial to the activities of ferric oxidase, NADH oxidase, succinate dehydrogenase, and many cytochromes. As such, iron sequestration is a major defense mechanism employed to ward off pathogens. To specifically sequester bacterial siderophores, the host typically employs lipocalin-2, which preferentially binds to catecholate moieties [29, 30]. Not surprisingly, iron and iron-siderophore transporters were found to be present in high numbers in the

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probiotic strains as well as the pathogens. In addition, TC family 1.B.14 includes outer membrane receptors that are involved in the uptake of iron-siderophore complexes and are found in lower numbers in *E. coli* K-12 and both *Salmonella*, than in the other strains. The probiotic and pathogenic *E. coli* contain more high-affinity inner membrane iron-siderophore uptake porters (TC class 3.A.1) than *E. coli* K-12 or *Salmonella* [31]. Interestingly, the numbers of outer membrane receptors and inner membrane ABC-type transporters coincide, suggesting that they co-evolved, as discussed previously [17].

The increased number of iron-siderophore transporters in probiotics, especially in *E. coli* Nissle, has been show to contribute to the ability of these organisms to outcompete *Salmonella* in the gut [32]. While *E. coli* and *Salmonella* pathogens both encode siderophore transporters that can evade the lipocalin-2 response, the probiotic *E. coli* also contain such systems (i.e., for ferrichrome and heme) in higher numbers [29, 30]. This, in turn, allows the probiotic strains to scavenge iron more efficiently than the remaining strains, thus allowing them to outcompete pathogens [33]. As hypothesized by Deriu et al., this may allow the probiotic *E. coli* to behave as a reserve immune system when the host's defenses are evaded [32].

With regards to the two *Salmonella* strains examined, CT18 contains more ABC transporters, which are ATP-dependent. Based on previous studies, this occurrence of ABC transporters in CT18 may reflect a greater dependence upon substrate-level phosphorylation (i.e., glycolysis) to generate energy, and consequently may have a greater anaerobic capacity than LT2 does [34, 35]. In contrast, LT2 contains more secondary carriers, which are dependent upon the proton-motive force (pmf). Conversely, the higher numbers of these transporters probably reflects the greater reliance of LT2 on an aerobic lifestyle because more energy is generated through oxidative phosphorylation (i.e., Krebs cycle, electron transport chains) [34, 35].

Not surprisingly, *E. coli* O83 and Nissle lack almost all of the pore-forming toxins that are found in the pathogens. Both probiotic strains contain cytotoxic fimbrial subunit transporters, though the relevance of these systems is not clear since *E. coli* strains produce dozens of fimbriae with a variety of functions in adhesion, biofilm formation, motility, conjugation, and virulence [36, 37]. Consistent with previous studies, *E. coli* Nissle contains an additional toxin, colicin V, which is toxic to other bacteria, but not to humans [13]. This may allow *E. coli* Nissle to antagonize pathogens, conferring upon it a competitive advantage over them.

In addition, only *E. coli* O157 and the *Salmonella* strains have IIITCP proteins. The EspB/D complex and other homologous pore-forming protein pairs are secreted into the host cell and oligomerize to form translocation pores at the site of contact, providing a route of entry for *E. coli* effector proteins to inhibit host phagocytosis by altering host cell cytoskeletal functions [38, 39]. Together with the adhesin, intimin, and its receptor, Tir, these proteins are typically involved in attaching-and-effacing (AE) mechanisms in enteropathogenic strains [40]. Therefore, the presence of the T3SS and the formation of an actin pedestal during AE lesion formation have become hallmarks of *E. coli* and *Salmonella* pathogenicity, and as

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such, the presence of a complete T3SS is only found in *E. coli* O157 and the *Salmonella* strains [4, 41].

The presence of other protein secretion systems in all nine strains examined shows very disparate patterns, as most strains contain many constituents of various systems, though some are incomplete and therefore presumably nonfunctional. This suggests that the common ancestor had several of these systems, but they were partially lost by some of their progeny. The T6SS has been shown to be important in antagonizing other bacteria and in use as a defense mechanism through "T6SS dueling" [42]. Studies have shown that intercellular T6SS dynamics involve targeting prey cells by translocation, or "injection" of effector proteins that puncture the prey's membrane [42]. The absence of a T6SS in both probiotic strains is noteworthy, as it was possible that *E. coli* O83 could have inhibited growth of pathogens via T6SS dueling. However, this observation is consistent with the previous finding that *E. coli* Nissle does not inhibit pathogens through physical contact [14].

Our studies have shown that a few virulence factors that are present in pathogenic *E. coli* and *Salmonella* strains are also present in the probiotic strains. These include increased numbers of high-affinity sugar and iron uptake systems. However, other virulence factors, such as toxins, T6SSs, and T3SSs, are not found in the probiotic strains. Previous studies had shown that many factors that contribute to pathogenesis have been acquired through horizontal gene transfer (HGT), and these are often found in pathogenicity islands on the chromosome [13]. The results provided by G-BLAST allow detailed comparative analyses, though there are shortcomings, such as its inability to infer HGT. Future efforts will seek to detect HGT and to integrate transporter proteomics with other genomic analyses. Based on the presented data, it is possible that some transporters will prove to be useful for diagnostic purposes or for genetically engineering probiotic strains.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Pitout JD. Extraintestinal Pathogenic Escherichia coli: A Combination of Virulence with Antibiotic Resistance. Front Microbiol. 2012; 3:9. [PubMed: 22294983]
2. Leimbach A, Hacker J, Dobrindt U. E. coli as an all-rounder: the thin line between commensalism and pathogenicity. Curr Top Microbiol Immunol. 2013; 358:3–32. [PubMed: 23340801]
3. Fierer J, Guiney DG. Diverse virulence traits underlying different clinical outcomes of Salmonella infection. J Clin Invest. 2001; 107:775–80. [PubMed: 11285291]
4. Coburn B, Grassl GA, Finlay BB. Salmonella, the host and disease: a brief review. Immunol Cell Biol. 2007; 85:112–8. [PubMed: 17146467]
5. Dougan G, Baker S. Salmonella enterica serovar Typhi and the pathogenesis of typhoid fever. Annu Rev Microbiol. 2014; 68:317–36. [PubMed: 25208300]

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6. Schaechter M. Escherichia coli and Salmonella 2000: the View From Here. EcoSal Plus. 2004; 1
7. Ventura M, O'Flaherty S, Claesson MJ, Turroni F, Klaenhammer TR, van Sinderen D, et al. Genome-scale analyses of health-promoting bacteria: probiogenomics. Nat Rev Microbiol. 2009; 7:61–71. [PubMed: 19029955]
8. Jonkers D, Penders J, Masclee A, Pierik M. Probiotics in the management of inflammatory bowel disease: a systematic review of intervention studies in adult patients. Drugs. 2012; 72:803–23. [PubMed: 22512365]
9. Backhed F, Ding H, Wang T, Hooper LV, Koh GY, Nagy A, et al. The gut microbiota as an environmental factor that regulates fat storage. Proc Natl Acad Sci U S A. 2004; 101:15718–23. [PubMed: 15505215]
10. Samuel BS, Gordon JI. A humanized gnotobiotic mouse model of host-archaeal-bacterial mutualism. Proc Natl Acad Sci U S A. 2006; 103:10011–6. [PubMed: 16782812]
11. Ventura M, Canchaya C, Tauch A, Chandra G, Fitzgerald GF, Chater KF, et al. Genomics of Actinobacteria: tracing the evolutionary history of an ancient phylum. Microbiol Mol Biol Rev. 2007; 71:495–548. [PubMed: 17804669]
12. Boirivant M, Strober W. The mechanism of action of probiotics. Curr Opin Gastroenterol. 2007; 23:679–92. [PubMed: 17906447]
13. Grozdanov L, Raasch C, Schulze J, Sonnenborn U, Gottschalk G, Hacker J, et al. Analysis of the genome structure of the nonpathogenic probiotic Escherichia coli strain Nissle 1917. J Bacteriol. 2004; 186:5432–41. [PubMed: 15292145]
14. Westendorf AM, Gunzer F, Deppenmeier S, Tapadar D, Hunger JK, Schmidt MA, et al. Intestinal immunity of Escherichia coli NISSLE 1917: a safe carrier for therapeutic molecules. FEMS Immunol Med Microbiol. 2005; 43:373–84. [PubMed: 15708311]
15. Hejnova J, Dobrindt U, Nemcova R, Rusniok C, Bomba A, Frangeul L, et al. Characterization of the flexible genome complement of the commensal Escherichia coli strain A0 34/86 (O83 : K24 : H31). Microbiology. 2005; 151:385–98. [PubMed: 15699189]
16. Azmatullah A, Qamar FN, Thaver D, Zaidi AK, Bhutta ZA. Systematic review of the global epidemiology, clinical and laboratory profile of enteric fever. J Glob Health. 2015; 5:020407. [PubMed: 26649174]
17. Tang F, Saier MH Jr. Transport proteins promoting Escherichia coli pathogenesis. Microb Pathog. 2014; 71-72:41–55. [PubMed: 24747185]
18. Saier MH Jr, Yen MR, Noto K, Tamang DG, Elkan C. The Transporter Classification Database: recent advances. Nucleic Acids Res. 2009; 37:D274–8. [PubMed: 19022853]
19. Reddy VS, Saier MH Jr. BioV Suite--a collection of programs for the study of transport protein evolution. FEBS J. 2012; 279:2036–46. [PubMed: 22568782]
20. Ikeda M, Arai M, Lao DM, Shimizu T. Transmembrane topology prediction methods: a re-assessment and improvement by a consensus method using a dataset of experimentally-characterized transmembrane topologies. In Silico Biol. 2002; 2:19–33. [PubMed: 11808871]
21. Zhai Y, Saier MH Jr. A web-based program (WHAT) for the simultaneous prediction of hydropathy, amphipathicity, secondary structure and transmembrane topology for a single protein sequence. J Mol Microbiol Biotechnol. 2001; 3:501–2. [PubMed: 11545267]
22. Saier MH Jr, Reddy VS, Tamang DG, Vastermark A. The transporter classification database. Nucleic Acids Res. 2014; 42:D251–8. [PubMed: 24225317]
23. Saier MH Jr. A functional-phylogenetic classification system for transmembrane solute transporters. Microbiol Mol Biol Rev. 2000; 64:354–411. [PubMed: 10839820]
24. Saier MH Jr, Reddy BL. Holins in bacteria, eukaryotes, and archaea: multifunctional xenologues with potential biotechnological and biomedical applications. J Bacteriol. 2015; 197:7–17. [PubMed: 25157079]
25. Reddy BL, Saier MH Jr. Properties and Phylogeny of 76 Families of Bacterial and Eukaryotic Organellar Outer Membrane Pore-Forming Proteins. PLoS One. 2016; 11:e0152733. [PubMed: 27064789]
26. Prestin K, Wolf S, Feldtmann R, Hussner J, Geissler I, Rimmbach C, et al. Transcriptional regulation of urate transportosome member SLC2A9 by nuclear receptor HNF4alpha. Am J Physiol Renal Physiol. 2014; 307:F1041–51. [PubMed: 25209865]

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27. Derrien M, van Hylckama Vlieg JE. Fate, activity, and impact of ingested bacteria within the human gut microbiota. Trends Microbiol. 2015; 23:354–66. [PubMed: 25840765]
28. Tremaroli V, Backhed F. Functional interactions between the gut microbiota and host metabolism. Nature. 2012; 489:242–9. [PubMed: 22972297]
29. Fischbach MA, Lin H, Liu DR, Walsh CT. How pathogenic bacteria evade mammalian sabotage in the battle for iron. Nat Chem Biol. 2006; 2:132–8. [PubMed: 16485005]
30. Flo TH, Smith KD, Sato S, Rodriguez DJ, Holmes MA, Strong RK, et al. Lipocalin 2 mediates an innate immune response to bacterial infection by sequestrating iron. Nature. 2004; 432:917–21. [PubMed: 15531878]
31. Anderson DS, Adhikari P, Nowalk AJ, Chen CY, Mietzner TA. The hFbpABC transporter from Haemophilus influenzae functions as a binding-protein-dependent ABC transporter with high specificity and affinity for ferric iron. J Bacteriol. 2004; 186:6220–9. [PubMed: 15342592]
32. Deriu E, Liu JZ, Pezeshki M, Edwards RA, Ochoa RJ, Contreras H, et al. Probiotic bacteria reduce salmonella typhimurium intestinal colonization by competing for iron. Cell Host Microbe. 2013; 14:26–37. [PubMed: 23870311]
33. Purschke FG, Hiller E, Trick I, Rupp S. Flexible survival strategies of Pseudomonas aeruginosa in biofilms result in increased fitness compared with Candida albicans. Mol Cell Proteomics. 2012; 11:1652–69. [PubMed: 22942357]
34. Paulsen IT, Nguyen L, Sliwinski MK, Rabus R, Saier MH Jr. Microbial genome analyses: comparative transport capabilities in eighteen prokaryotes. J Mol Biol. 2000; 301:75–100. [PubMed: 10926494]
35. Paulsen IT, Sliwinski MK, Saier MH Jr. Microbial genome analyses: global comparisons of transport capabilities based on phylogenies, bioenergetics and substrate specificities. J Mol Biol. 1998; 277:573–92. [PubMed: 9533881]
36. Chahales P, Thanassi DG. Structure, Function, and Assembly of Adhesive Organelles by Uropathogenic Bacteria. Microbiol Spectr. 2015; 3
37. Giltner CL, Nguyen Y, Burrows LL. Type IV pilin proteins: versatile molecular modules. Microbiol Mol Biol Rev. 2012; 76:740–72. [PubMed: 23204365]
38. Condry DL, Nilles ML. Introduction to Type III Secretion Systems. Methods Mol Biol. 2017; 1531:1–10. [PubMed: 27837477]
39. Iizumi Y, Sagara H, Kabe Y, Azuma M, Kume K, Ogawa M, et al. The enteropathogenic E. coli effector EspB facilitates microvillus effacing and antiphagocytosis by inhibiting myosin function. Cell Host Microbe. 2007; 2:383–92. [PubMed: 18078690]
40. Croxen MA, Finlay BB. Molecular mechanisms of Escherichia coli pathogenicity. Nat Rev Microbiol. 2010; 8:26–38. [PubMed: 19966814]
41. Schmidt MA. LEEways: tales of EPEC, ATEC and EHEC. Cell Microbiol. 2010; 12:1544–52. [PubMed: 20716205]
42. Basler M, Ho BT, Mekalanos JJ. Tit-for-tat: type VI secretion system counterattack during bacterial cell-cell interactions. Cell. 2013; 152:884–94. [PubMed: 23415234]

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Highlights

- *E. coli* pathogens, probiotics and commensals were examined for transport proteins
- Consistent patterns were observed for pathogens versus probiotics
- Intercellular versus extracellular strains could also be distinguished

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Table 1

Overview of the nine Gram-negative enteric bacterial strains included in this study and their basic traits.

Organism	Strain	Abbreviation	Accession no.	Genome size (Mbp)	Total proteins	Total transport proteins	Host location	Pathological condition
	K-12 MG1655	K-12	NC_000913.3	4.64	4140	842	Extracellular	None
	O83:H1	O83	NC_017634.1	4.75	4429	855	Extracellular	None
	O6:K5:H1 (Nissle 1917)	Nissle	CP007799.1	5.44	4821	873	Extracellular	None
Escherichia coli	O157:H7	O157	NC_002695.1	5.50	5204	874	Extracellular	Hemorrhagic colitis
	UMN026	UMN	NC_011751.1	5.20	4819	910	Extracellular	Urinary tract infection
	CFT073	CFT	AE014075.1	5.23	5016	886	Intracellular	Urinary tract infection and pyelonephritis
	ABU 83792	ABU	CP001671.1	5.13	4796	898	Intracellular	Bacteremia
Salmonella enterica	CT18 (Typhi)	CT18	NC_003198.1	4.81	4111	821	Intracellular	Typhoid fever
	LT2 (Typhimurium)	LT2	NC_003197.1	4.86	4451	862	Intracellular	Enterocolitis

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Table 2

Overview of the *E. coli* and *Salmonella* transport protein analyses based on TC subclass.

	# Transport proteins									%								
TC subclass and description	**K-12**	**O83**	**Nissle**	**O157**	**UMN**	**CFT**	**ABU**	**CT18**	**LT2**	**K-12**	**O83**	**Nissle**	**O157**	**UMN**	**CFT**	**ABU**	**CT18**	**LT2**
1.A, α-type channels	29	34	33	34	35	33	38	33	34	3.4	4.0	3.8	3.9	3.9	3.7	4.2	4.0	3.9
1.B, β-barrel porins	64	79	86	76	78	74	86	62	62	7.6	9.2	9.9	8.7	8.6	8.4	9.6	7.6	7.2
1.C, Pore-forming toxins	6	2	4	12	7	10	11	8	8	0.7	0.2	0.5	1.4	0.9	1.1	1.2	1.0	0.9
1.E, Holins	9	11	7	9	10	7	9	11	13	1.1	1.3	0.8	1.0	1.1	0.8	1.0	1.3	1.5
2.A, Porters (uniporters, symporters, antiporters)	273	269	267	265	272	280	278	225	254	32.5	31.5	30.6	30.4	29.9	31.6	31.0	27.4	29.5
2.C, Ion-gradient-driven energizers	3	4	4	3	5	3	5	5	5	0.4	0.5	0.5	0.3	0.6	0.3	0.6	0.6	0.6
3.A, P-P-bond-hydrolysis-driven transporters	226	265	269	249	257	250	243	267	270	26.9	31.0	30.8	28.5	28.3	28.2	27.1	32.5	31.3
3.B, Decarboxylation-driven transporters	2	0	0	2	2	2	0	6	6	0.2	0.0	0.0	0.2	0.2	0.2	0.0	0.7	0.7
3.D, Oxidoreduction-driven transporters	50	35	37	49	48	40	38	36	39	5.9	4.1	4.2	5.6	5.3	4.5	4.2	4.4	4.5
4.A, Phosphotransfer-driven group translocators	43	50	55	47	53	53	54	34	42	5.1	5.8	6.3	5.4	5.8	6.0	6.0	4.1	4.9
4.B, Nicotinamide ribonucleoside uptake transporters	2	2	2	2	2	3	2	2	2	0.2	0.2	0.2	0.2	0.2	0.3	0.2	0.2	0.2
4.C, Acyl-CoA ligase-coupled transporters	2	2	2	2	2	2	3	3	3	0.2	0.2	0.2	0.2	0.2	0.2	0.3	0.4	0.3
4.D, Polysaccharide synthase exporters	4	3	3	3	3	4	4	6	6	0.5	0.4	0.3	0.3	0.3	0.5	0.4	0.7	0.7
5.A, Transmembrane two-electron transfer carriers	29	26	27	28	30	28	26	28	29	3.4	3.0	3.1	3.2	3.3	3.2	2.9	3.4	3.4
5.B, Transmembrane one-electron transfer carriers	4	0	0	2	5	2	2	3	3	0.5	0.0	0.0	0.2	0.6	0.2	0.2	0.4	0.3
8.A, Auxiliary transport proteins	21	16	15	21	22	21	23	17	15	2.5	1.9	1.7	2.4	2.4	2.4	2.6	2.1	1.7
9.A, Recognized transporters of unknown biochemical mechanism	8	10	10	7	11	10	10	12	9	1.0	1.2	1.1	0.8	1.2	1.1	1.1	1.5	1.0
9.B, Putative transport proteins	66	47	52	62	66	64	66	63	62	7.8	5.5	6.0	7.1	7.3	7.2	7.3	7.7	7.2
Total	841	855	873	873	908	886	898	821	862	100	100	100	100	100	100	100	100	100

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Table 3

Overview of transport proteins in *E. coli* and *Salmonella* based on predicted substrate specificities.

	# Transport proteins									%								
Substrate category	**K-12**	**O83**	**Nissle**	**O157**	**UMN**	**CFT**	**ABU**	**CT18**	**LT2**	**K-12**	**O83**	**Nissle**	**O157**	**UMN**	**CFT**	**ABU**	**CT18**	**LT2**
Anions	40	48	50	43	47	45	47	38	38	4.9	5.7	5.8	5.0	5.3	5.2	5.4	4.7	4.5
Cations	113	105	105	114	123	109	109	113	112	13.8	12.5	12.2	13.4	13.9	12.6	12.5	14.1	13.2
Electrons	36	29	30	33	43	33	36	37	41	4.4	3.5	3.5	3.9	4.8	3.8	4.1	4.6	4.8
Water	1	1	1	1	1	1	1	0	0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.0
Amines	22	22	21	22	20	21	21	25	24	2.7	2.6	2.4	2.6	2.3	2.4	2.4	3.1	2.8
Amino acids and derivatives	74	75	78	72	68	72	72	78	80	9.0	8.9	9.1	8.5	7.7	8.3	8.2	9.7	9.4
Carboxylates	41	43	42	38	39	46	41	30	43	5.0	5.1	4.9	4.5	4.4	5.3	4.7	3.7	5.1
Drugs	30	39	41	34	45	38	46	41	42	3.7	4.6	4.8	4.0	5.1	4.4	5.3	5.1	5.0
Nonselective	20	19	19	21	19	21	21	15	15	2.4	2.3	2.2	2.5	2.1	2.4	2.4	1.9	1.8
Nucleobases and nucleosides	22	24	25	25	26	25	25	18	20	2.7	2.9	2.9	2.9	2.9	2.9	2.9	2.2	2.4
Siderophores	24	31	38	26	30	32	34	16	19	2.9	3.7	4.4	3.1	3.4	3.7	3.9	2.0	2.2
Sugars	70	80	84	67	74	74	77	48	50	8.5	9.5	9.8	7.9	8.3	8.6	8.8	6.0	5.9
Sugar alcohols	13	15	14	11	14	14	14	12	14	1.6	1.8	1.6	6.9	10.3	9.9	11.1	9.4	10.1
Sugar derivatives	15	15	12	14	15	14	14	17	23	1.8	1.8	1.4	1.6	1.7	1.6	1.6	2.1	2.7
Vitamins	11	12	12	11	11	11	11	10	11	1.3	1.4	1.4	1.3	1.2	1.3	1.3	1.2	1.3
DNA	12	15	13	16	14	13	14	17	20	1.5	1.8	1.5	1.9	1.6	1.5	1.6	2.1	2.4
Lipids	15	13	14	13	14	15	15	13	13	1.8	1.5	1.6	1.5	1.6	1.7	1.7	1.6	1.5
Polysaccharides	23	25	25	18	25	26	24	21	21	2.8	3.0	2.9	2.1	2.8	3.0	2.7	2.6	2.5
Proteins and peptides	125	131	129	159	136	142	126	127	139	15.2	15.6	15.0	18.7	15.3	16.4	14.4	15.8	16.4
Unknown	113	97	105	114	122	113	127	128	122	13.8	11.6	12.2	13.4	13.9	13.1	14.5	15.9	14.4
Total	820	839	858	852	887	865	875	804	847	100	100	100	100	100	100	100	100	100

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Table 4

Occurrence of various metabolite transport systems in extracellular versus intracellular strains of *E. coli* and *Salmonella*. The first five strains listed live extracellularly while the last four live intracellularly in the host.

TCID	K-12	O83	Nissle	O157	UMN	CFT	ABU	CT18	LT2	Specific Substrate
1.B.17.1.4	0	0	0	1	1	0	0	0	0	Proteins, polysaccharides, drugs
2.A.1.1.3	1	0	0	1	0	0	0	0	0	Xylose
2.A.1.5.3	0	0	0	1	1	0	0	0	0	Sucrose
2.A.1.15.2	1	0	0	1	1	0	0	0	0	3-(3-hydroxyphenyl)propionate
2.A.1.17.1	1	0	0	1	1	0	0	0	0	Cyanate
2.A.1.20.3	1	0	0	0	1	0	0	0	0	Arabinose
2.A.2.3.6	0	0	0	1	1	0	0	0	0	Cellobiose
2.A.7.1.11	0	0	0	0	1	0	0	0	0	Drugs
2.A.7.19.2	0	0	1	0	1	0	0	0	0	Allantoin, uric acid, xanthine
2.A.8.1.1	1	0	1	0	0	0	0	0	0	Gluconate
2.A.23.1.4	0	1	1	0	0	0	0	0	0	Cystine, selenocystine
2.A.26.1.2	1	1	1	1	0	0	0	0	0	Isoleucine, valine
2.A.41.2.10	1	0	0	1	1	0	0	0	0	Nucleosides
2.A.61.1.2	1	0	0	1	1	0	0	0	0	C_4-dicarboxylates
2.A.68.1.1	1	0	0	1	1	0	0	0	0	p-aminobenzoyl-glutamate
3.A.1.1.28	0	1	1	0	0	0	0	0	0	Raffinose, stachyose
3.A.1.1.34	0	1	1	0	0	0	0	0	0	Arabinose
3.A.1.1.41	0	1	1	0	0	0	0	0	0	Trehalose
3.A.1.1.44	0	1	0	0	0	0	0	0	0	Maltose
3.A.1.2.14	1	1	1	0	0	0	0	0	0	Arabinose
3.A.1.2.20	0	1	1	0	0	0	0	0	0	Glucose
3.A.1.12.4	0	1	1	0	0	0	0	0	0	Osmolytes
3.A.1.12.10	0	1	1	0	0	0	0	0	0	Glycine betaine, choline, acetylcholine, carnitine, proline betaine
3.A.1.17.2	0	1	1	0	0	0	0	0	0	Aromatic sulfonates
3.A.1.26.1	0	1	1	0	0	0	0	0	0	Vitamin B_{12}

TCID	K-12	O83	Nissle	O157	UMN	CFT	ABU	CT18	LT2	Specific Substrate
3.A.1.106.5	0	1	1	1	0	0	0	0	0	Norfloxacin, tetracycline, DAPI
4.A.1.1.10	0	0	0	1	1	0	0	0	0	α-Glucoside
4.A.1.2.3	1	1	0	1	1	0	0	0	0	β-Glucoside
4.A.2.1.6	1	0	0	1	0	0	0	0	0	Mannose
2.A.1.3.14	0	0	0	0	0	0	0	1	1	Drugs
2.A.1.4.2	0	0	0	0	0	1	0	1	1	Phosphoglycerates
2.A.1.6.7	0	0	0	0	0	0	0	1	1	Citrate, tricarballylate
2.A.1.14.9	0	0	0	0	0	0	0	1	1	p-hydroxyphenylacetate
2.A.3.3.19	0	0	0	0	0	0	0	1	1	Histamine
2.A.3.13.2	0	0	0	0	0	0	0	1	1	Ceftriaxone
2.A.7.22.2	0	0	0	0	0	0	0	1	1	Undecaprenyl phosphate-α-aminoarabinose
2.A.10.1.3	0	0	0	0	0	0	0	1	1	2-keto-3-deoxygluconate
2.A.25.1.9	0	0	0	0	0	0	0	1	1	Amino acids
2.A.47.3.4	0	0	0	0	0	0	0	1	1	Citrate
2.A.66.1.22	0	0	0	0	0	0	0	1	1	Quinolones
2.A.80.1.1	0	0	0	0	0	1	1	1	1	Tricarboxylates
2.A.117.1.3	0	0	0	0	0	0	0	1	1	Chlorhexidine
3.A.1.3.29	0	0	0	0	0	0	0	1	1	Histidine, arginine, lysine
3.A.1.11.5	0	0	0	0	0	0	0	1	1	2-aminoethyl phosphonate
3.A.1.12.15	0	0	0	0	0	0	0	1	1	Glycine, betaine, choline
3.A.1.24.2	0	0	0	0	0	0	0	1	1	Methionine
4.A.2.1.19	0	0	0	0	0	0	0	1	1	Tagatose
4.A.6.1.17	0	0	0	0	0	0	0	1	1	Glucosaminate
4.D.2.1.9	0	0	0	0	0	0	0	1	1	Glycosyl residues
4.D.3.1.6	0	0	0	0	0	0	0	1	1	Glycosyl residues

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Table 5

Occurrence of secretion system components in *E. coli* and *Salmonella*. Transporters marked in yellow are present in all *E. coli* strains; those marked in green are probiotic-specific; those marked in red are pathogen-specific; those marked in purple are *Salmonella*-specific.

Family	TCID	Function	K-12	O83	Nissle	O157	UMN	CFT	ABU	CT18	LT2
T1SS	3.A.1.105.4	Drug exporter 1 (4 components)	0	4	4	0	0	0	0	0	0
	3.A.1.109.4	Protein exporter 1 (4 components)	0	0	0	2	2	2	0	2	2
	3.A.1.110.1	Protein exporter 2 (4 components)	0	0	3	0	0	3	2	0	0
	3.A.1.113.3	Peptide 3 exporter (4 components)	1	1	1	1	1	1	1	1	1
T2SS GSP	3.A.5.1.1	SEC-SRP complex (7 components)	7	7	7	7	7	7	7	7	7
	3.A.6.2.1	Flagellar protein export complex (10 components)	10	10	10	10	10	10	10	10	10
MTB	3.A.15.1.1	Pullulanase secretion system (12 components)	7	10	10	0	5	7	7	1	2
	3.A.15.2.1	Pilin secretion/fimbrial assembly system (9 components)	3	2	2	4	2	3	2	2	3
T3SS	3.A.6.1.1	Type III secretion system complex (12 components)	1	0	0	13	5	1	0	11	11
	3.A.6.2.1	Type III secretion system complex (10 components)	10	10	10	10	10	10	10	10	10
T4SS	3.A.7.3.1	Pertussis toxin exporter (8 components)	0	0	0	0	5	0	0	0	0
	3.A.7.7.1	Trs DNA transfer protein complex (15 components)	2	3	2	2	1	2	2	1	1
	3.A.7.9.1	Icm/Dot protein secretion system (26 potential components)	0	1	1	0	0	0	0	0	0
	3.A.7.11.1	Type IV betaproteobacterial DNA secretion system (20 potential components)	3	3	0	3	0	1	0	4	0
	3.A.7.14.1 and 2	Type IV (conjugal DNA-protein transfer) (15 components)	2	2	4	2	2	3	3	3	3
T6SS	3.A.23.1.1	T6SS VasA-L (14 components)	14	11	6	25	18	21	10	3	4
	3.A.23.2.1	T6SS EvpA-P (16 components)	1	4	3	2	0	5	0	2	5
	3.A.23.3.1	T6SS TssA-G (12 components)	0	0	0	0	5	0	3	0	0
	3.A.23.4.1	T6SS TssA-H (11 components)	0	0	0	0	0	0	0	1	1
	3.A.23.5.1	T6SS TssB-M (12 components)	0	0	0	0	1	0	1	0	0

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Table 6

Occurrence of iron and iron-siderophore transporters in *E. coli* and *Salmonella*. Transporters marked in yellow are present in all *E. coli* strains; those marked in green are probiotic-specific; those marked in red are pathogen-specific; those marked in purple are *Salmonella*-specific. Polarity of transport is indicated in columns 3 and 4 where an X indicates that the transport process (uptake and/or efflux) can occur in the indicated direction.

TCID	Substrate Transported	Uptake	Efflux	K-12	O83	Nissle	O157	UMN	CFT	ABU	CT18	LT2
1.B.14.1.1	Fe^{3+}-coprogen	X		1	1	1	1	1	1	1	0	1
1.B.14.1.2	Fe^{3+}-ferrichrome	X		1	1	1	1	1	1	1	0	1
1.B.14.1.3	Fe^{3+}-enterobactin	X		0	0	1	0	0	1	1	1	1
1.B.14.1.4	Fe^{3+}-catecholate	X		1	1	1	1	1	1	1	1	1
1.B.14.1.9	Fe^{3+}-catecholate	X		1	1	1	1	1	1	1	0	0
1.B.14.1.11	Fe^{3+}-coprogen	X		0	0	0	0	0	1	0	0	0
1.B.14.1.13	Fe^{3+}	X		0	0	1	1	1	1	1	0	0
1.B.14.1.15	Fe^{3+}-ferrichrome	X		0	0	1	0	0	0	0	0	0
1.B.14.1.20	Fe^{3+}-citrate	X		1	0	1	0	1	0	1	0	0
1.B.14.1.22	Fe^{3+}-ferrichrome	X		1	1	1	1	1	1	1	1	1
1.B.14.1.24	Fe^{3+}-enterobactin	X		0	1	1	1	0	1	0	0	0
1.B.14.2.2	Heme	X		0	0	1	0	0	1	1	0	0
1.B.14.2.14	Heme	X		0	1	1	1	1	1	1	0	0
1.B.14.8.7	Fe^{3+}-yersiniabactin	X		0	0	0	0	1	0	1	0	0
1.B.14.9.3	Fe^{3+}-ferrichrome	X		0	0	1	0	1	1	1	0	0
1.B.14.9.4	Fe^{3+}-yersiniabactin, bacteriocin	X		1	1	0	1	0	0	0	0	0
1.B.14.10.1	Heme	X		0	1	0	0	0	0	0	0	0
2.A.1.38.1	Enterobactin		X	1	1	1	1	0	1	1	0	0
2.A.1.38.3	Enterobactin	X		0	0	0	0	0	0	0	1	1
2.A.1.57.3	Fe^{3+} chelator	X		0	1	1	0	1	1	1	0	0
2.A.4.7.1	Zn^{2+}, Cd^{2+}, Hg^{2+}, Fe^{2+}		X	1	1	1	1	1	1	1	1	1
2.A.5.5.1	Fe^{2+}, Co^{2+}, Mn^{2+}, Cd^{2+}, Zn^{2+}	X		1	1	1	1	1	1	1	1	1
2.A.55.3.1	Mn^{2+}, Fe^{2+}, Cd^{2+}, Co^{2+}, Zn^{2+}, Ni^{2+}	X		0	1	1	1	1	1	1	1	1
2.A.108.2.3	Fe^{2+}	X		0	3	1	1	3	1	3	0	0

TCID	Substrate Transported	Uptake	Efflux	K-12	O83	Nissle	O157	UMN	CFT	ABU	CT18	LT2
3.A.1.14.1	Fe^{3+}-citrate	X		4	0	4	0	4	0	4	0	0
3.A.1.14.2	Fe^{3+}-enterobactin	X		4	4	4	4	4	4	4	4	4
3.A.1.14.3	Fe^{3+}-hydroxamate, albomycin	X		3	3	3	3	3	3	3	3	3
3.A.1.14.5	Heme	X		0	0	0	0	0	0	0	0	1
3.A.1.14.6	Fe^{3+}-vibriobactin/enterobactin	X		0	1	1	1	0	1	0	0	0
3.A.1.14.8	Fe3+-vibrioferrin	X		0	1	1	0	0	0	0	0	0
3.A.1.14.15	Fe^{3+}-bacillibactin	X		0	1	1	1	0	0	0	0	0
3.A.1.14.18	Heme	X		0	3	3	3	3	3	3	0	0
3.A.1.15.7	Mn^{2+}, Fe^{2+}	X		0	4	4	0	4	4	4	4	4
3.A.1.21.1	Fe^{3+}-yersiniabactin	X		0	2	2	0	2	2	2	0	0
3.A.1.106.7	Salmonchellin, enterobactin		X	0	0	1	0	0	1	1	1	1
3.A.1.107.3	Heme		X	3	3	3	3	3	3	3	3	3
3.A.1.139.2	Fe^{2+}		X	1	2	1	1	2	0	2	2	2
9.A.8.1.1	Fe^{2+}	X		1	1	1	1	1	1	1	1	1
9.A.8.1.7	Fe^{2+}	X		0	0	0	0	0	0	0	1	0
9.A.8.1.9	Fe^{2+}	X		0	0	0	0	0	0	0	1	0
9.A.8.1.10	Fe^{2+}	X		1	0	0	0	0	0	0	1	1
9.B.14.2.3	Heme		X	1	1	1	1	1	1	1	1	1

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Table 7

Occurrence of toxins in *E. coli* and *Salmonella*. Toxins marked in yellow are present in all *E. coli* strains; those marked in green are probiotic-specific; those marked in red are pathogen-specific.

TCID	Family	Function	K-12	O83	Nissle	O157	UMN	CFT	ABU	CT18	LT2
1.C.1.2.2	Colicin	Colicin E1	0	0	0	0	0	1	1	0	0
1.C.10.1.1	HlyE	Hemolysin, HlyE	1	0	0	1	1	1	1	1	0
1.C.11.1.3	RTX toxin	Hemolysin, HlyA	0	0	0	0	0	1	1	0	0
1.C.31.1.3	Colicin V	Colicin V precursor, CcaV	0	0	1	0	0	0	1	0	0
1.C.36.1.1	IIITCP	T3SS: pore-forming complex EspBD	0	0	0	2	0	0	0	0	0
1.C.36.3.1	IIITCP	T3SS: pore-forming complex IpaBCD	0	0	0	0	0	0	0	0	1
1.C.36.3.2	IIITCP	T3SS: pore-forming complex SipBD	0	0	0	0	0	0	0	2	2
1.C.36.5.1	IIITCP	T3SS: pore-forming complex SseBCD	0	0	0	0	0	0	0	3	3
1.C.36.6.1	IIITCP	T3SS: pore-forming complex EspAD	0	0	0	1	0	0	0	0	0
1.C.54.1.1	Shiga toxin B	Shiga toxin B, St-B	0	0	0	1	0	0	0	0	0
1.C.57.3.2	Clostridial cytotoxin	Pore formation; necrosis in host, Cnf	0	0	0	0	0	0	1	0	0
1.C.57.3.3	Clostridial cytotoxin	Pore formation; necrosis in host, Cnf	0	0	0	1	0	0	0	0	0
1.C.75.1.1	S-PFT	Hemolysin, ShlA	0	0	0	1	0	1	0	0	0
1.C.80.1.1	Cytotoxic major fimbrial subunit (MrxA)	Adhesive fimbriae (pore formation)	0	0	1	0	1	1	1	0	0
1.C.80.1.2	Cytotoxic major fimbrial subunit (MrxA)	Adhesive fimbriae (pore formation)	1	1	1	1	1	1	1	0	0
1.C.80.1.3	Cytotoxic major fimbrial subunit (MrxA)	Adhesive fimbriae (pore formation)	1	1	1	1	1	1	1	0	0
1.C.80.1.4	Cytotoxic major fimbrial subunit (MrxA)	Adhesive fimbriae (pore formation)	1	0	0	1	1	1	1	0	0
1.C.82.1.1	HP2-20	Pore formation	1	0	0	1	1	1	1	1	1
1.C.113.1.1	Hly III	Hemolysin, Hly III	1	0	0	1	0	1	0	0	0
1.C.113.1.10	Hly III	Hemolysin, YqfA	0	0	0	0	1	0	1	1	1

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Table 8

Occurrence of various metabolite transporters exclusive to either *Salmonella* Typhi or Typhimurium.

TCID	CT18	LT2	Specific Substrate
2.A.1.14.1	0	1	Glucarate
2.A.1.14.3	0	1	Tartrate
2.A.2.3.7	0	1	Arabinosides
2.A.3.7.1	0	1	Glutamate
2.A.7.17.1	0	1	Phenylalanine, tryptophan
2.A.13.1.3	0	1	C_4-dicarboxylates
2.A.21.3.10	0	1	Sialic acid
2.A.24.1.1	0	1	Citrate
2.A.40.2.1	0	1	Purines
2.A.78.2.3	0	1	Amino acids
4.A.6.1.19	0	4	Glucoselysine, fructoselysine
1.B.35.1.7	1	0	Carbapenem?
3.A.1.3.22	1	0	Glutamic acid
3.A.1.3.23	1	0	Lysine, arginine, histdine, alanine, valine
3.A.1.101.2	2	0	Capsular Polysaccharide