JOURNAL OF BACTERIOLOGY, Jan. 2009, p. 388–393 Vol. 191, No. 1
0021-9193/09/$08.00+0 doi:10.1128/JB.01052-08

A Genomic Island of an Extraintestinal Pathogenic *Escherichia coli* Strain Enables the Metabolism of Fructooligosaccharides, Which Improves Intestinal Colonization[▽]§

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Received 29 July 2008/Accepted 20 October 2008

Prebiotics such as fructooligosaccharides (FOS) are increasingly being used in some countries for improving human and animal health and as an alternative to antibiotic growth promoters in animals, with various degrees of success. It has been observed that FOS stimulate the proliferation of probiotic bacteria and, at the same time, decrease the population of bacteria associated with disease. This observation assumes that pathogenic bacteria do not metabolize FOS and, therefore, lose their competitive advantage over beneficial bacteria. Here we present evidence that some pathogenic *Escherichia coli* strains can metabolize FOS and show that this property helps the bacterium colonize the intestine. These findings highlight the potential risk that a high level of prebiotic usage could lead to the emergence of well-adapted pathogenic strains that metabolize prebiotic substances.

A prebiotic is a selectively fermented ingredient which allows specific changes in the composition of and/or activity in the gastrointestinal microbiota which confer health benefits on the host (37). Many carbohydrates, including short-chain fructooligosaccharides (scFOS), are reported to be prebiotic. scFOS are polymers of fructose units with the generic structure α-D-Glu-(1-2)-(β-D-Fru-1-2-)*n*, where *n* is between 2 and 4. FOS are low-energy ingredients with a taste similar to that of sucrose. They are used commercially in food products and nutritional supplements. In the intestines of humans, rats, horses, pigs, and chicks, FOS stimulate growth of probiotic bacteria, and some studies have found that they inhibit the growth of pathogenic bacteria such as *Salmonella enterica* serovar Typhimurium and *Escherichia coli* (4, 7, 33, 42). Genes involved in the assimilation of FOS by probiotic bacteria, such as lactic acid bacteria, have been identified (3, 21, 39), but so far they have not been found in pathogenic bacteria.

Extraintestinal pathogenic *E. coli* strains are normal inhabitants of the guts of humans and warm-blooded animals. They are the major cause of extraintestinal infections, being the principal agent of urinary tract infections, the second most common agent of neonatal meningitis, and one of the two most common agents of bacteremia (25). They are also responsible for intra-abdominal, soft tissue, and respiratory tract infections (38). The latter infection is particularly found in poultry and often leads to air sacculitis, perihepatitis, and pericarditis, as well as other syndromes, such as osteomyelitis (2, 16).

In order to colonize different niches, *E. coli* has acquired new functions by horizontal gene transfer (29). Much of this horizontally transferred DNA is clustered into genomic islands (17). We previously identified a pathogenicity island, named AGI-3 (GenBank accession number AY857617), in the chromosome of an extraintestinal avian-pathogenic strain of *E. coli*, BEN2908. AGI-3 shows a modular structure composed of five loci bound by mobility-related genes (10). Three genes of the first locus are related to carbon metabolism and are involved in the virulence of the strain for chickens at an early stage of infection. The second locus (termed locus 2), comprising genes aec41 to aec47, is also thought to be involved in the assimilation of carbohydrates. Homology searches indicate that it codes for a transcriptional regulator of the LacI family (Aec47), a sugar transporter of the major facilitator superfamily (Aec46), two proteins of unknown functions (Aec42 and Aec44), a fructokinase (Aec41), and two glycoside hydrolases (Aec43 and Aec45) (Fig. 1) (10). Glycoside hydrolases (EC 3.2.1.) are a widespread group of enzymes that hydrolyze the glycosidic bond between two or more carbohydrates or between a carbohydrate and a noncarbohydrate moiety. They have been grouped into families based on their overall amino acid sequence similarities (12). The two glycoside hydrolases encoded by locus 2 genes belong to family 32 in the carbohydrate-active enzyme database (http://www.cazy.org). Enzymes of this family are functionally related and are involved in the hydrolysis of glycosidic bonds of fructose polymers, such as inulin and fructans. To be metabolized by *E. coli*, fructose has to be phosphorylated (18). *E. coli* does not usually possess cytoplasmic fructokinase. Fructose enters the cell via a phosphotransferase system (PTS), and phosphorylation is concomitant with membrane transport (28). The fact that locus 2 putatively encodes a fructokinase and glycoside hydrolases hydrolyzing fructose-containing polymers suggests that the carbohydrate transported and metabolized via locus 2 contains fructose units.

This study shows that locus 2 of the pathogenicity island AGI-3 is involved in scFOS metabolism. By comparing the abilities of the wild-type strain BEN2908 and its isogenic mutant, which is unable to metabolize scFOS, to colonize the

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§ Supplemental material for this article may be found at http://jb.asm.org/.

▽ Published ahead of print on 31 October 2008.

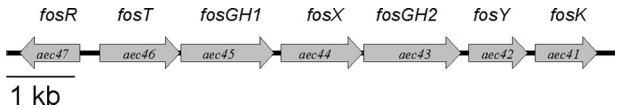

FIG. 1. Gene organization of the *fos* locus of *E. coli* strain BEN2908. The locus comprises *fosR* (aec47, putative regulator), *fosT* (aec46, putative sugar transporter), *fosGH1* (aec45, putative glycoside hydrolase), *fosX* (aec44, unknown function), *fosGH2* (aec43, putative glycoside hydrolase), *fosY* (aec42, unknown function), and *fosK* (aec41, putative fructose kinase).

intestine, we demonstrate that locus 2 contributes to the intestinal fitness of the pathogenic *E. coli* strain BEN2908.

MATERIALS AND METHODS

Bacterial strains and culture conditions. The main bacterial strains and plasmids used in this study are listed in Table 1. *E. coli* strain BEN2908, O2:K1:H5 (Fim^+ Iut^+ $IbeA^+$ AGI-3^+), is a nalidixic acid-resistant derivative of strain MT78 which was isolated from the trachea of a chicken with a respiratory infection (10, 15, 20). *E. coli* strain BEN2908 belongs to the phylogenetic group B2 (32). For cloning experiments, *E. coli* strains XL1-Blue and MG1655 were used (5, 8). For phenotypic and genotypic studies, a total of 133 strains were used; they included 72 strains from the ECOR collection (*E. coli* reference collection containing 61 fecal isolates from humans and animals, 10 urinary tract infection isolates, and 1 asymptomatic bacteriuria isolate from humans), 34 strains from humans with extraintestinal syndromes (meningitis or septicemia), and 27 strains from chickens that tested positive for the first locus of AGI-3 (4 nonpathogenic and 23 extraintestinal pathogenic strains) (10, 34). Strains were routinely grown in Luria-Bertani (LB) medium at 37°C with agitation. When necessary, kanamycin or ampicillin was added at a final concentration of 50 μg/ml or 100 μg/ml, respectively.

For cloning experiments, plasmid pGEM-T easy vector was used (Promega).

Construction of the *fosT* mutant and cloning of the *fos* locus. The *fosT* knockout mutant was constructed by using the method developed by Datsenko and Wanner and as applied previously (10, 14). In brief, after recombination, *fosT* was deleted and replaced by a kanamycin resistance cassette obtained by PCR amplification of plasmid pKD4 by using primers cat147 and cat148, which contain extensions homologous to the 5′ and 3′ ends of *fosT* (see Table S1 in the supplemental material). The replacement of *fosT* was confirmed by PCR using the primer pairs px1/cat162 and px2/cat156 (see Table S1 in the supplemental material), which made it possible to detect the left and right arms of the insertion, respectively. To clone the *fos* locus, the whole locus (9,027 bp) was amplified from strain BEN2908 by PCR using the primer pair cat154/cat155 (see Table S1 in the supplemental material) and Herculase enhanced DNA polymerase (Stratagene). The resulting PCR product was inserted into the PGEM-T easy vector (Promega). The ligation product was electroporated into competent XL1-Blue, prepared as described by Tung and Chow (44). Recombinant plasmids were analyzed by restriction analysis using PstI and BamHI (Promega) and by PCR using primer pairs cat167/cat168 and cat163/cat164 (see Table S1 in the supplemental material), allowing the amplification of the junctions between *fosY* and *fosK* and between *fosGH2* and *fosX*, respectively. A recombinant pGEM::*fos* plasmid was then introduced by electroporation into electrocompetent MG1655 cells.

Growth monitoring. For growth monitoring experiments, overnight LB cultures were centrifuged, washed twice, and resuspended in the same volume of M9 minimal medium (31). The strains were then cultured in triplicate at 37°C in 100-well, sterile, covered microplates (Labsystems, Helsinki, Finland). Each well contained 300 μl of M9 medium supplemented with either 0.5% scFOS (Profeed P95; Beghin Meiji, France), 0.2% glucose (Sigma), 5 mM kestose (GF2; Wako Chemicals GmbH, Germany), 5 mM nystose (GF3; Wako Chemicals GmbH, Germany), or 5 mM fructofuranosyl nystose (GF4; Wako Chemicals GmbH, Germany). The plates were incubated in a Microbiology Reader Bioscreen C apparatus (Labsystems, Helsinki, Finland), and the turbidity from 405 to 600 nm was measured every 30 min, after shaking. For analysis of the FOS^+ phenotype, overnight LB cultures were centrifuged, washed twice, and resuspended in the same volume in M9 minimal medium. The strains were then cultured overnight in 5 ml of M9 medium supplemented with 0.5% scFOS or 0.2% glucose. A negative control experiment was performed using M9 medium with no carbon source.

Detection of *fos* genes in *E. coli* isolates. The presence of *fos* genes in *E. coli* isolates was analyzed by PCR using primer pairs Rorf10/Forf10 and cat49/cat50, allowing the detection of the *fosGH2* gene and the *fosR-fosT* intergenic region, respectively (see Table S1 in the supplemental material). PCRs were performed using a 25-μl volume containing 500 nM of the forward and reverse primers, 200 μM of each deoxynucleoside triphosphate (Finnzymes, Ozyme, France), 1 U of *Taq* DNA polymerase (New England Biolabs Inc.), and 2 mM $MgCl_2$ in a PCR buffer containing 1 mM KCl, 1 mM $(NH_4)_2SO_4$, 200 μM $MgSO_4$, 0.1% Triton X-100, 2 mM Tris-HCl (pH 8.8) (New England Biolabs Inc.), and 5 μl of DNA template prepared by the boiling method (40). The PCR conditions were as follows: initial denaturation at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 50°C for 30 s, and extension at 72°C for 1 min/kb. A final extension step at 72°C for 7 min was included. Reactions were performed in a PerkinElmer thermocycler (GeneAmp 9700; Applied Biosystems).

Intestinal colonization of chickens. Axenic strain PA12 White Leghorn chicks were obtained from the INRA infectiology platform by using the method described by Le Bars (30). Specific-pathogen-free (SPF) chickens were obtained by orally inoculating 8-day-old axenic chicks with a complex bacterial inoculum consisting of 0.5 ml of a 1/10 suspension of feces from adult SPF hens (6). The *E. coli* strains in this inoculum were all antibiotic sensitive. The housing, husbandry, and slaughtering conditions conformed to European Union guidelines for the care and use of laboratory animals. The experimental protocol was approved by the regional ethical committee under number CL2007-43. Animals were reared in isolators, fed ad libitum on a commercial diet sterilized by gamma irradiation (poultry starter HPS; Dietex, France), and supplied with autoclaved tap water or 0.5% autoclaved scFOS throughout the experiment. After 18 h of food starvation, eight 12-day-old axenic chicks or eight 12-day-old SPF chicks were fed with 0.5 ml of a mixture of equal numbers of bacteria of the wild-type strain BEN2908 Nal^r and the mutant derivative BEN2908Δ*fosT* Kan^r Nal^r (each approximately at 5×10^6 CFU) in LB medium. Fresh fecal samples were collected on days 1, 2, 3, 6, 8, 10 or 13, 15, and 20 or 21 postgavage. Fecal samples were weighed and then homogenized in distilled water (9 ml/g of feces). Viable *E. coli* cells were counted by plating 10-fold dilutions in sterile saline on Drigalski agar (Bio-Rad), with nalidixic acid at 40 μg/ml (selections of the wild-type and Δ*fosT* strains) or with kanamycin at 50 μg/ml (selection of the Δ*fosT* strain). The numbers of fecal CFU were calculated per gram of feces. The detection threshold was 100 CFU/g of feces. The numbers of wild-type bacterial CFU (BEN2908

TABLE 1. Strains and plasmids used in this study

Strain or plasmid	Relevant characteristic(s)	Reference or source
E. coli strains		
BEN2908	Extraintestinal pathogenic strain; O2:K1:H5 (Nal^r Fos^+ Fim^+ Iut^+ $IbeA^+$ AGI-3^+), avian origin	15
BEN2908Δ*fosT*	Isogenic deletion mutant of BEN2908; Kan^r Fos^-	This study
MG1655	Nonpathogenic, Fos^-, human origin	5
XL1-Blue	*recA1 endA1 gyrA96 thi-1 hsdR17 supE44 relA1 lac* [F′ *proAB lacI*^{q}*Z*ΔM15 Tn*10* (Tet^r)]	Stratagene
Plasmids		
pKD4	*oriR*γ Amp^r Kan^r	14
pKD46	*oriR101 repA101*(ts) *araBp-gam-bet-exo* Amp^r	14
pGEM-T easy vector	Amp^r	Promega
pGEM::*fos*	pGEM containing the whole *fos* locus	This study

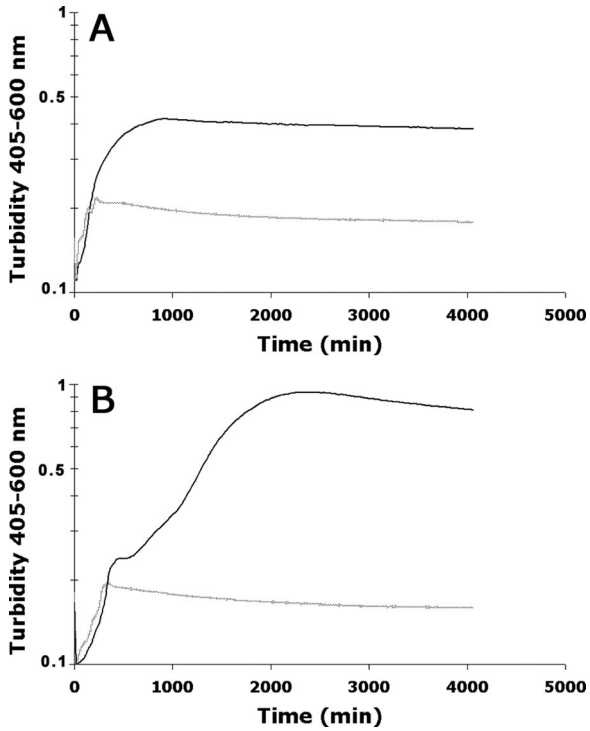

FIG. 2. The metabolism of scFOS is mediated by locus 2 of AGI-3. *E. coli* strains BEN2908 (solid black curve) and BEN2908Δ*aec46* (dashed gray curve) (A) or MG1655 (dashed gray curve) and MG1655/pGEMT::*aec41-47* (solid black curve) (B) were grown in M9 medium containing 0.5% scFOS at 37°C with agitation.

Nal^r) were calculated by subtracting the number of kanamycin-resistant bacteria from the number of nalidixic-resistant bacteria. Competition indices (CI) were calculated similarly to the method of Freter et al. by using BEN2908 as the reference strain [CI = (number of BEN2908 CFU/number of BEN2908Δ*fosT* CFU recovered from chicken feces)/(number of BEN2908 CFU/number of BEN2908Δ*fosT* CFU present in the initial inoculum)] (19). By definition, a CI of >1 indicates outcompetition of the mutant strain (BEN2908Δ*fosT*) by the wild-type reference strain (BEN2908). A CI equal to 1 indicates no difference in colonization, and a CI of <1 indicates outcompetition of the wild-type reference strain (BEN2908) by the mutant (BEN2908Δ*fosT*). In all colonization experiments, we verified that the kanamycin-resistant *E. coli* population was unable to metabolize scFOS. To that end, 5 ml of LB medium containing kanamycin at 50 μg/ml was inoculated with 100 μl of each fecal sample and incubated overnight at 37°C with agitation. Overnight LB cultures were centrifuged, washed twice with M9 minimal medium, and resuspended in the same volume of M9 minimal medium. Five milliliters of M9 minimal medium supplemented with 0.5% scFOS was then inoculated with 100 μl of the washed culture and incubated overnight at 37°C with agitation. No growth was observed for any of the fecal samples tested.

Statistical analysis. Statistical analyses of CI were performed by using the Mann-Whitney U test. Exact *P* values were calculated with StatXact software (version 5.0; Cytel Inc., Cambridge, MA).

RESULTS

Involvement of locus 2 of the pathogenicity island AGI-3 in the metabolism of FOS. Since in silico analysis of locus 2 of the pathogenicity island AGI-3 of BEN2908 suggested that it is involved in the metabolism of a sugar containing fructose units, we tested the ability of BEN2908 to grow in minimal medium containing different fructose polymers as the sole carbon source. We found that BEN2908 was not able to grow in minimal medium containing sucrose, raffinose, or inulin but thrived in the presence of scFOS (data not shown and Fig. 2A). This indicates that locus 2 of AGI-3 could play a role in the metabolism of scFOS.

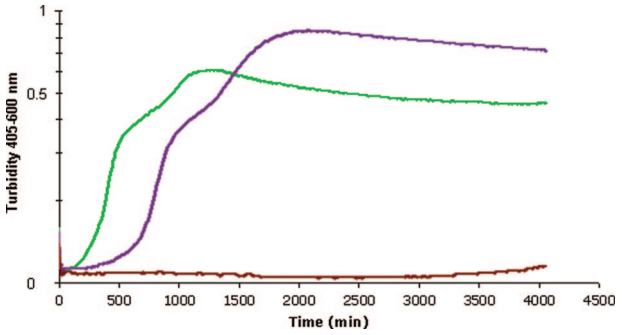

FIG. 3. Preferential use of the shorter-chain FOS. *E. coli* strain BEN2908 was grown in M9 medium containing either 5 mM GF2 (green curve), 5 mM GF3 (purple curve), or 5 mM GF4 (red curve) at 37°C with agitation.

To prove that locus 2 is involved in scFOS metabolism, we first constructed a derivative of strain BEN2908 by replacing the aec46 gene, encoding the putative scFOS transporter, with a kanamycin resistance cassette. We then tested the ability of the mutant strain to grow in a minimal medium with scFOS as the sole carbon source. Figure 2A indicates that BEN2908Δ*aec46* is unable to grow in this medium. Secondly, we introduced a recombinant plasmid carrying the whole locus 2 in the nonpathogenic *E. coli* strain MG1655, which is unable to metabolize scFOS. The recombinant MG1655 strain was now able to grow with scFOS as the sole carbon source (Fig. 2B). Taken together, these two experiments prove that the second locus of AGI-3 is involved in scFOS metabolism. Accordingly, we renamed locus 2 the *fos* locus and the individual genes of locus 2 *fosR* (aec47, putative regulator), *fosT* (aec46, scFOS transporter), *fosGH1* (aec45, putative glycoside hydrolase), *fosX* (aec44, unknown function), *fosGH2* (aec43, putative glycoside hydrolase), *fosY* (aec42, unknown function), and *fosK* (aec41, putative fructose kinase) (Fig. 1).

Different sugar polymers belong to the family of scFOS, depending on their numbers of fructose units. To identify which type of scFOS is consumed by strain BEN2908, we monitored the growth of BEN2908 in M9 minimal medium supplemented with either 5 mM of kestose (2 units of fructose [GF2]), nystose (3 units of fructose [GF3]), or fructofuranosylnystose (4 units of fructose [GF4]). It was found that BEN2908 metabolized GF2 and GF3 at the same rate but consumed GF2 earlier than GF3. GF4 did not allow the growth of BEN2908 (Fig. 3).

Effect of the *fos* locus on intestinal colonization. FOS are naturally found in many plants, such as wheat (13), and consequently are present in chicken feed and in the intestine, the primary habitat of extraintestinal pathogenic *E. coli*. We thus examined the effect of the *fos* locus on the ability of *E. coli* BEN2908 to colonize the chicken intestine. To that end, *E. coli* BEN2908Δ*fosT* (formerly BEN2908Δ*aec46*) and its wild-type parent were fed together in equal amounts to axenic chickens (5×10^6 CFU of each strain/chicken), and the proportion of each in feces was monitored. Figure 4A indicates that the

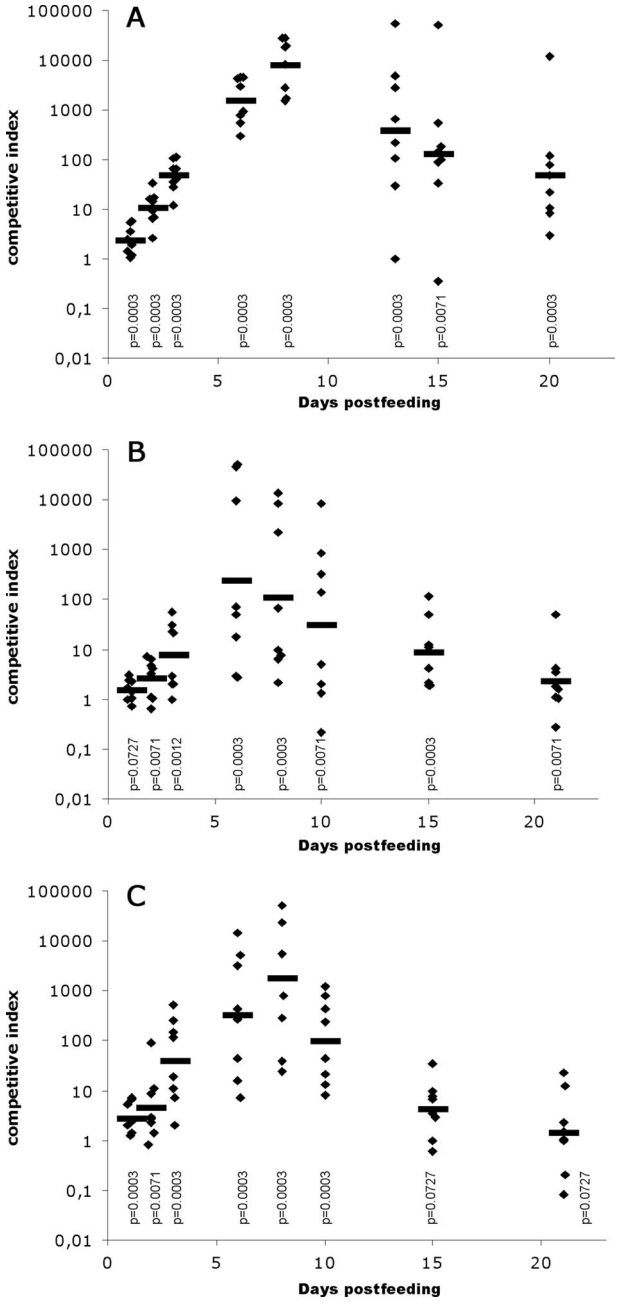

FIG. 4. Competition between *E. coli* strains BEN2908 and BEN2908Δ*fosT* to colonize the chicken intestine. Axenic chickens (A), SPF chickens (B), or SPF chickens receiving a dietary supplement of 0.5% scFOS in drinking water (C) were fed with strains BEN2908 and BEN2908Δ*fosT* together (each at 5×10^6 CFU/chicken). The proportion of each strain in animal feces was monitored over time, and CI were calculated. Horizontal bars indicate the geometric means of CI, and diamonds indicate individual CI. Statistical analyses were conducted using the Mann-Whitney U test, measuring the difference between CI in feces samples and in the inoculum. The calculated *P* values are presented, with values below 0.05 considered significant.

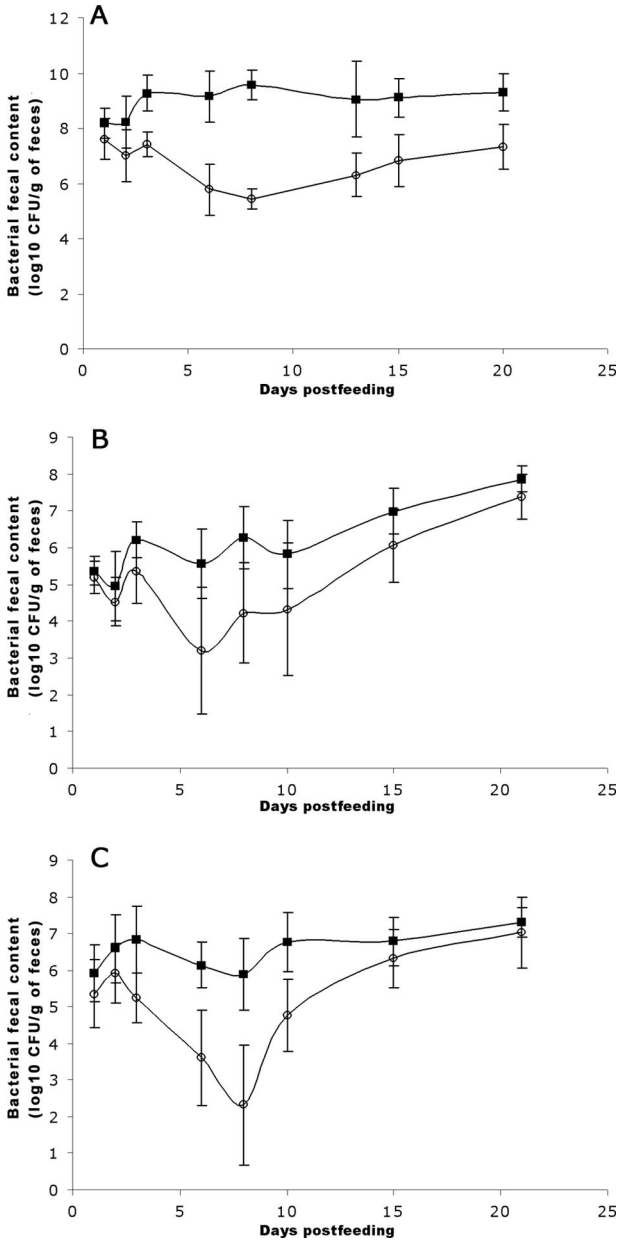

FIG. 5. Monitoring of chicken intestine colonization by *E. coli* strain BEN2908 (filled squares) and its mutant derivative BEN2908Δ*fosT* (open circles). Axenic chickens (A), SPF chickens (B), or SPF chickens receiving a dietary supplement of 0.5% scFOS in drinking water (C) were fed with strains BEN2908 and BEN2908Δ*fosT* together (each at 5×10^6 CFU/chicken). The presence of each strain in the feces was monitored over time. Bars represent standard errors of the $\log_{10}$ (mean number of CFU/g of feces) for each set of eight chickens.

mutant strain was strongly outcompeted by the wild-type strain. The mean CI increased up to day 8 postfeeding (mean CI of 8,078) and then decreased, although it was still substantial at day 20 (mean CI of 48). This decrease was not a consequence of a global *E. coli* population decrease due to the development of mucosal immunity, since more than 10^9 CFU/g of feces were still present at day 20 (Fig. 5A). These results indicate that the *fos* locus is beneficial during the initial stage of colonization and less important during the maintenance stage.

To mimic more natural conditions, we repeated this experiment with SPF chickens which have a complex biota, contain-

ing commensal *E. coli* among others bacteria. Even when this complex biota is found in the intestine, our findings indicate that strain BEN2908 is perfectly able to colonize the intestine (10^8 CFU/g feces at day 21 postfeeding) (Fig. 5B) and to outcompete the Δ*fosT* mutant, reaching lower but still highly competitive levels (Fig. 4B). This indicates that scFOS metabolism provides selective advantages for strain BEN2908 within the highly competitive gastrointestinal tract at the expense of a similar strain that does not metabolize it. The fact that mean CI were lower in the presence of a complex microbiota than in axenic chickens may suggest that other bacteria of the flora are more efficient FOS degraders than strain BEN2908.

Dietary supplements of FOS have been used at poultry farms to improve the health and performance of poultry (36). We therefore also evaluated the impact of increasing the scFOS content of the chicken diet (0.5% in drinking water) on the capacity of strains BEN2908 and BEN2908Δ*fosT* to colonize the intestines of SPF chickens (Fig. 4C). Although the mean CI was lower at day 8, when the chicken diet was not supplemented with scFOS, than when it was supplemented with 0.5% scFOS, this difference was not found to be statistically significant (Fig. 4B and C). This result indicates that dietary supplementation of scFOS does not increase the ability of strain BEN2908 to colonize the intestine.

Other *E. coli* strains are able to metabolize scFOS. To determine whether the metabolism of scFOS is a property unique to BEN2908, we performed a rapid survey of various *E. coli* strains, testing the 72 strains of the *E. coli* reference collection (ECOR), 27 *E. coli* strains of avian origin that previously tested positive for the first locus of AGI-3, and 34 strains isolated from humans with extraintestinal syndromes (10, 34). Phenotypic analysis indicated that 4 of the 72 strains of the ECOR collection are able to metabolize FOS; these 4 strains were all isolated from healthy animals (ECOR7 from an orangutan, ECOR23 from an elephant, ECOR31 from a leopard, and ECOR32 from a giraffe). Only one of these strains, ECOR31, was positive when the presence of *fosGH2* and overlapping *fosT* and *fosR* fragments were tested using PCR. Among the 27 strains of avian origin possessing the first locus of AGI-3 (24 extraintestinal pathogenic strains and 3 nonpathogenic strains), 17 were able to grow with scFOS as the sole carbon source and 9 were PCR test positive (1 of these 9 strains is nonpathogenic). Of the 34 strains isolated from humans with extraintestinal syndromes (meningitis or septicemia), 2 were able to grow in the presence of scFOS but were PCR test negative. Thus, some commensal and pathogenic *E. coli* strains also possess the ability to metabolize scFOS via the *fos* locus described here. However, other genes which have not yet been identified are probably implicated in scFOS metabolism in *E. coli*.

DISCUSSION

FOS is used as a food additive in Europe and Japan for its bifidogenic effect on human colonic endogenous biota, among other things. FOS is considered a protective nutrient, leading to a decrease in the pathogenic population. It has been suggested that probiotic bacteria in the intestinal tract, such as lactobacilli and bifidobacterial species, preferentially use FOS (26). There is increasing evidence that lactobacilli and bifidobacteria develop antimicrobial activities which are involved in the host's gastrointestinal defense system (41). These antimicrobial activities are due partly to the production of lactic acid and an increase in short-chain fatty acid production, resulting in a lower pH in the large intestine which could suppress or displace undesirable or pathogenic bacteria, such as *Clostridium perfringens*, *Salmonella enterica* serovar Typhimurium, and *E. coli* (1, 33, 43). This study demonstrates that, like many probiotic bacteria of the biota, some pathogenic *E. coli* strains are able to metabolize FOS. This metabolism of FOS helps the strain colonize the intestine, especially in the initial stages. This growth advantage for a pathogenic *E. coli* strain could suppress the beneficial effect provided by the ingestion of FOS.

E. coli is a very versatile bacterium with high genetic diversity. The plasticity of its genome is mainly the result of gene rearrangement within the genome and of the acquisition of novel traits by horizontal transfer (plasmids, bacteriophages, transposons, or genomic islands). The FOS metabolic genes identified in this study are located in the pathogenicity island AGI-3, whose characteristics are linked to the mobility of the island (presence of direct repeats flanking the island and of an integrase gene) (10, 24). This suggests that AGI-3 has the capacity to be mobile, a property which could contribute to the emergence of the FOS metabolic trait among pathogenic *E. coli* strains and other pathogenic bacteria of the intestinal biota under selective pressure for FOS utilization. The dissemination of the *fos* locus would lead to a global increase in the intestinal fitness of the recipient strains and in the risk to public health, thus negating the expected positive effects provided by the use of FOS.

The *fos* locus presented here has never been reported before, and its origin is still unknown. Various genes involved in FOS metabolism have previously been described to occur in lactic acid bacteria. A gene cluster encoding an ATP-dependent binding cassette (ABC)-type transporter involved in the uptake of scFOS has been reported in *Lactobacillus acidophilus* (3). *Lactobacillus paracasei* transports scFOS via an ABC transport system or a PTS (21, 26). FOS uptake in *Bifidobacterium longum* may be mediated via an ABC-type transporter (23, 35). All of these systems are different from the *fos* locus of strain BEN2908. The homolog closest (54% identity) to the BEN2908 FosT transporter is a non-PTS sugar permease called FruP which has been identified in *Bacillus megaterium* but for which the transported substrate has not been identified (9). The homologs closest to the glycoside hydrolases FosGH1 and FosGH2 are a β-fructofuranosidase from *Bifidobacterium breve* (41% identity) and a glycoside hydrolase from *Bacteroides thetaiotaomicron* (28% identity), respectively (39, 46).

The digestive tracts of herbivores differ structurally from those of carnivores with regard to the site of cellulose digestion. The large intestine of birds consists of a shorter colon than that of mammals and typically have a pair of ceca. This leads to less efficient recovery of the nutrients from feedstuffs (45). Despite these anatomical and physiological differences between birds and mammals, the composition of the microbiota from the gastrointestinal tracts of chickens is largely similar to the human complex microbial ecosystem, although individual differences in the human microbiota have been found (11, 22, 27). Furthermore, numerous studies have shown

that *E. coli* strains (and particularly strain BEN2908 used in this study) isolated from chickens and humans are genetically similar (32). Our findings suggest that also in mammals pathogenic *E. coli* strains which have acquired the capacity to metabolize scFOS have a fitness advantage in the intestine, increasing the risk to human health under FOS pressure.

ACKNOWLEDGMENTS

We are grateful to Beghin-Meiji (Neuilly/Seine, France) for its generous donation of Profeed P95. We thank Annie Brée and Nathalie Lallier for their excellent assistance in the practical aspects of the work and the staff of the experimental platform for the care of the animals. We thank Roland Quentin and Eric Oswald for providing some strains.

The work was funded by ERA-NET PathoGenoMics (grant ANR-06-PATHO-002-01).

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