

MINIREVIEW

Role of Nonhost Environments in the Lifestyles of *Salmonella* and *Escherichia coli*

Mollie D. Winfield and Eduardo A. Groisman*

Howard Hughes Medical Institute, Department of Molecular Microbiology, Washington University
School of Medicine, St. Louis, Missouri 63110

The gram-negative bacterial species *Salmonella enterica* and *Escherichia coli* are members of the family *Enterobacteriaceae* that spend a good part of their lives as residents of animal hosts. *S. enterica* is the etiologic agent of gastroenteritis and typhoid fever in humans (88), whereas *E. coli* is most commonly known as a commensal of the lower intestine of mammals, although pathogenic variants also exist (77). The animal host is believed to be the primary habitat of these two enteric species (86), which are genetically endowed to do well in this environment. For example, *Salmonella* has genes that mediate invasion of and survival within host cells, including genes that promote resistance to different microbicidal host products (62, 88). Likewise, the *E. coli* genome encodes proteins that mediate resistance to acid pH as well as growth on lactose, which is critical for a commensal of mammals (11, 57).

In this review, we discuss whether *Salmonella* and *E. coli* live in stable, dividing populations in nonhost environments and whether such environments constitute dead ends for these species (e.g., as a consequence of residing in the vertebrate lower intestine, whose contents are regularly excreted [86, 102]). In addition, we examine the role that genes specific to *Salmonella* and *E. coli* play in the different abilities of these species to proliferate outside animal hosts.

DO *SALMONELLA* AND *E. COLI* EXIST IN A VBNC STATE?

The host environment provides *Salmonella* and *E. coli* with a warm constant temperature, as well as high concentrations of free amino acids and sugars, which are conducive to bacterial growth (86). Once excreted from an animal host, *Salmonella* and *E. coli* find themselves battling for survival, facing limited nutrient availability, osmotic stress, large variations in temperature and pH, and predation (61, 83, 86). It has been proposed that bacteria can survive such stressful conditions by entering a viable but nonculturable (VBNC) state.

The VBNC hypothesis describes an apparent dormant state in which bacterial cells are metabolically active but cannot be cultured by known laboratory methods (78). It has been suggested that bacteria enter this state in response to harsh envi-

ronmental conditions, such as a temperature change, high salinity, or nutrient deprivation (78, 82, 93). The controversy surrounding the VBNC concept involves whether bacterial cells can be resuscitated out of the dormant state and can start dividing in the external environment, which in the case of human enteric bacteria may constitute a public health concern.

Nutrient addition and temperature upshift are reportedly effective methods for rescuing bacterial populations from the VBNC state (78, 82, 93), but they may simply support the growth of a small number of culturable cells that survive the stress conditions and are not detected because of limitations of laboratory cultivation methods (13, 78). Thus, the apparent resuscitation of nonculturable cells may result from dispersion of clumps of culturable cells or from the response of undetected culturable cells to the new growth conditions, either of which would give the illusion of recovery (12). If one accepts that viability means “the ability of a single cell to attain a population discernible by the observer” (15), then the use of staining techniques to determine cell viability may not provide an accurate measurement of whether cells are alive (12); if this is true, few studies have actually detected viable cells emerging from the VBNC state.

Raw sewage is often discharged into low-temperature marine and aquatic environments, raising the question of whether human enteric pathogens (i.e., *Salmonella*) and indicator organisms (i.e., *E. coli*) are able to adapt to and persist in these extreme environments. Exposure of *Salmonella* and *E. coli* to polar marine conditions in Antarctica for 54 to 56 days altered their physiology to a state characteristic of VBNC cells. Although no viable colonies were observed after 54 days, the level of bacterial respiration was 90% of the initial levels after nutrient addition at day 46 (93). A *Salmonella* environmental isolate from the Potomac River in Washington, D.C., was found to be in the VBNC state following dilution into a laboratory microcosm containing sterilized Potomac River water. While the metabolic activity remained constant, the bacterial count decreased to zero after 4 days and resuscitation by nutrient addition to the river water on day 21 was unsuccessful (82), indicating that the cells may not have been viable.

Proponents of the VBNC hypothesis have suggested that bacterial species, particularly pathogens, pose a health risk when they are present in a dormant state in the environment because of the likelihood of resuscitation upon access to an animal host (78, 82, 93). However, evidence against recovery of VBNC cells brings into question the premise that dormant

* Corresponding author. Mailing address: Department of Molecular Microbiology, Howard Hughes Medical Institute, Washington University School of Medicine, 660 S. Euclid, Campus Box 8230, St. Louis, MO 63110. Phone: (314) 362-3692. Fax: (314) 747-8228. E-mail: groisman@borcim.wustl.edu.

pathogens are capable of causing disease. For example, seawater and UV-C radiation induce in *Salmonella* the VBNC state, which is characterized by a lack of culturability, 95% respiratory activity, and unrecoverable virulence in mice (16). Likewise, VBNC *S. enterica* serovar Oranienburg detected in dried processed squid was avirulent in BALB/c mice, and virulence was detected only in mice treated with morphine (4), which suppresses the mouse immune system. This raises the possibility that the virulence phenotype observed in morphine-treated mice is not due to resuscitation from the vegetative state but rather is the result of a bacterial product, such as lipopolysaccharide, which is present in VBNC organisms.

Enteropathogenic *E. coli* exposed to seawater and sunlight for 26 h (i.e., unculturable, yet metabolically active) was positive for enterotoxin in a rabbit intestinal loop assay (76). Nonetheless, the presence of enterotoxin does not provide strong evidence for the VBNC hypothesis, given that a toxin may remain stable in otherwise dead bacterial cells. Moreover, if VBNC pathogens do pose a health risk, one would expect to find a greater occurrence of outbreaks of waterborne bacterial diseases in developed nations (13). Recovery from a VBNC state occurs rarely, if at all, and long-term contamination appears to result from regular deposition of bacteria from human and animal sources, which may be the case in developing countries.

***E. COLI* HAS A LOW RATE OF SURVIVAL OUTSIDE ANIMAL HOSTS**

E. coli resides in the lower intestine of warm-blooded animals (92), an environment that provides a vast supply of nutrients for bacterial growth (22, 71, 86, 87). The doubling time of *E. coli* in this primary habitat has been estimated to be 2 days (86). It has been suggested that one-half of the total *E. coli* population resides in the primary habitat of the host and one-half is in the external environment (i.e., the secondary habitat) (86). According to this notion, *E. coli* grows and divides in its primary habitat, but it has a net negative rate of growth in the secondary habitat, with half-lives of approximately 1 day in water (29), 1.5 days in sediment (35), and 3 days in soil (101). These estimates imply that *E. coli* does not live in nonhost environments but that continuous bulk transfer from human and animal sources maintains a stable population outside animal hosts (86).

The genetic diversity of the *E. coli* population in individual hosts has been attributed to turnover of both resident strains (i.e., strains that persist for months to years) and transient strains (i.e., strains that persist for days to weeks) as a result of ingestion of contaminated food or water (19, 91). Hosts typically acquire *E. coli* during birth (9) and subsequently maintain one or two resident strains and three or four transient strains, all of which appear to change over time (2, 18, 19, 55, 89, 90). However, the mechanism of turnover of the host intestinal flora in the absence of antibiotic treatment remains unclear, as experimental implantation of new strains into healthy subjects is difficult (33, 34, 89, 90). Furthermore, the vast majority of ingested strains fail to colonize, as reflected by the relatively constant intestinal composition (90). Indeed, the cycle time of *E. coli* through a host has been estimated to range from 26 h to 66 years (85).

Survival of *E. coli* in the secondary habitat requires the ability to overcome low nutrient availability and wide temperature fluctuations. *E. coli* populations decline rapidly in freshwater microcosms (47, 59); however, addition of readily usable carbon sources, as well as removal of competitor microflora, allows growth, suggesting that *E. coli* is unable to acquire and compete for nutrients under starvation conditions (59).

The *E. coli* populations found in the secondary habitat are apparently maintained by constant arrival of microorganisms from the primary habitat (86), as a lack of nutrients and harsh ecological conditions prevent *E. coli* from sustaining a dividing population outside the animal host. This model implies that *E. coli* does not live in nonhost environments and that its presence in such locations results from excretion of waste by animal hosts. This is the logic behind the use of *E. coli* as the indicator organism for environmental fecal contamination (i.e., as an indicator species, *E. coli* is assumed not to be a permanent resident of soil and water) (49). Cumulatively, the results of experiments suggest that there is a low probability that *E. coli* will colonize a new host and that this is largely due to its relatively low survival rate in nonhost environments (Fig. 1B).

However, are there nonhost environments that mimic the physical and chemical milieu of the colon? If so, do *E. coli* populations live in such ecological niches as members of the natural flora?

***E. COLI* LIVES IN TROPICAL FRESHWATER AND SOIL**

As in the mammalian host environment, nutrients in tropical ecosystems are maintained at high concentrations, and together with constant warm air, soil, and water temperatures, this provides an ideal habitat for survival, growth, and proliferation of *E. coli* (Fig. 1C). Indeed, high concentrations of *E. coli* have been found in numerous tropical locations in the absence of known fecal sources (49). For example, the waters of Puerto Rico are highly contaminated with human waste, yet at sampling sites upstream of known sewage drainage points there are large numbers of fecal coliforms, similar to the numbers at sites downstream (49). Likewise, the number of viable *E. coli* cells in the upper one-third of the Mameyes River in Puerto Rico (in a cloud rain forest) shows a strong positive correlation with water temperature and nutrient concentrations (17). The total bacterial count found at this unspoiled tropical location exceeds by twofold the total count reported for the polluted, temperate Anacostia River in Washington, D.C. High respiration rates and extended survival suggest that tropical waters harbor natural populations of *E. coli* (17). However, studies performed with a pure culture of an *E. coli* strain that was not isolated from the Mameyes River showed that the size of the population decreased by 90% after 12 days, raising the possibility that a natural isolate in the region may be genetically adapted for increased survival in this environment (49). In spite of this, the survival time and the percentage of physiologically active cells (55 to 90%) suggest that *E. coli* is able to grow in a tropical freshwater environment (49, 60).

Water sources in uninhabited rain forests provide elevated concentrations of nutrients for thriving microbial communities in the absence of human pollution (81). Epiphyte species living in trees 15 m above the ground, such as bromeliads, form large cups with their leaves, which collect rainwater and runoff, thus

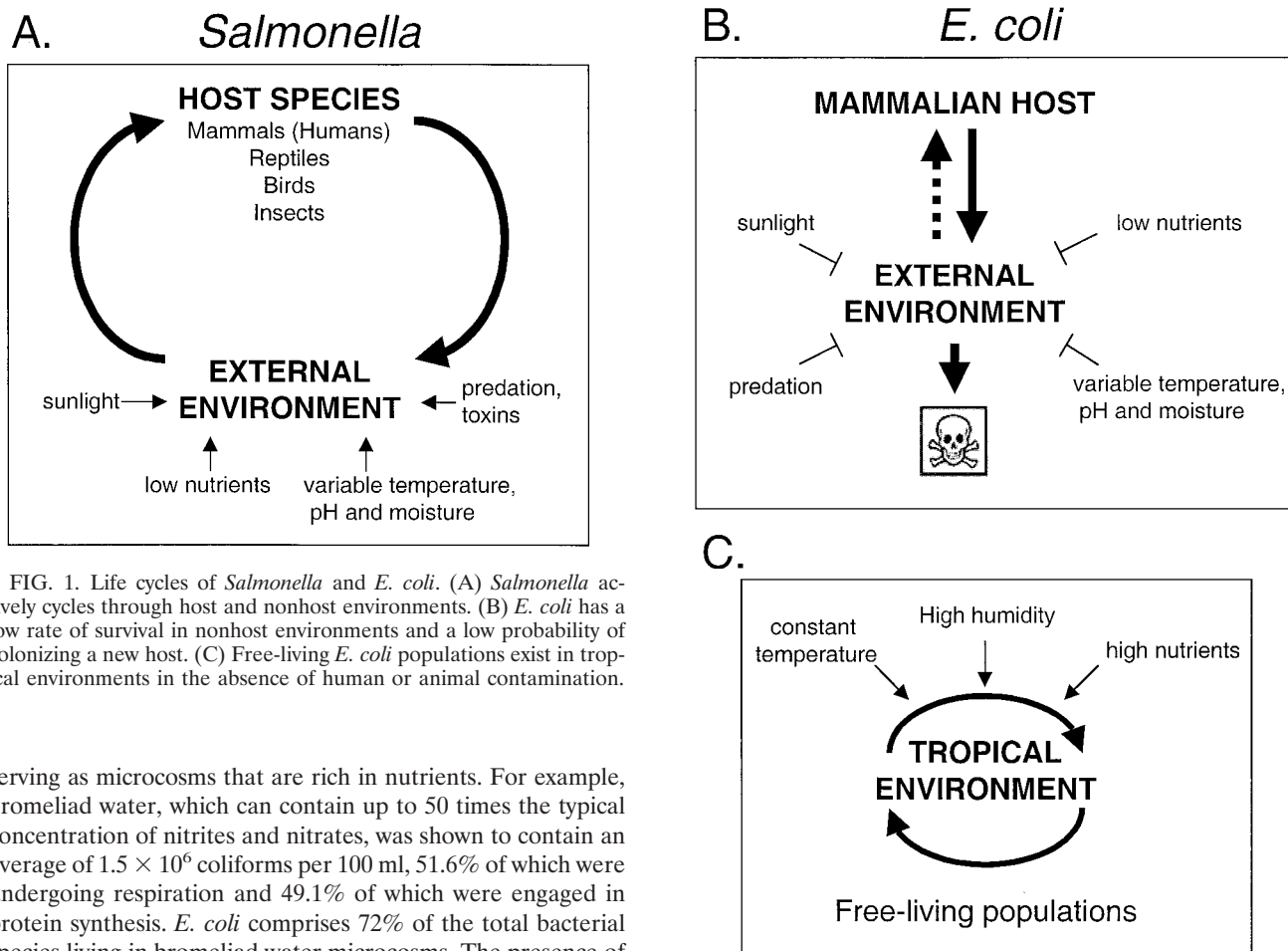


FIG. 1. Life cycles of *Salmonella* and *E. coli*. (A) *Salmonella* actively cycles through host and nonhost environments. (B) *E. coli* has a low rate of survival in nonhost environments and a low probability of colonizing a new host. (C) Free-living *E. coli* populations exist in tropical environments in the absence of human or animal contamination.

serving as microcosms that are rich in nutrients. For example, bromeliad water, which can contain up to 50 times the typical concentration of nitrites and nitrates, was shown to contain an average of 1.5×10^6 coliforms per 100 ml, 51.6% of which were undergoing respiration and 49.1% of which were engaged in protein synthesis. *E. coli* comprises 72% of the total bacterial species living in bromeliad water microcosms. The presence of active *E. coli* populations in bromeliad water at different forest elevations throughout the year suggests that these populations did not originate from the small population of Puerto Rican birds or tree-climbing mammals (81).

Natural *E. coli* populations are not limited to tropical freshwater. The soil of the North Fork of the New River in Ft. Lauderdale, Fla., is a major source of *E. coli* (97). This subtropical riverbank environment is characterized by warm and humid conditions with cyclic periods of wet and dry weather, which are conducive to *E. coli* growth (26, 97). While the presence of *E. coli* in this soil correlates with storm sewer flooding due to heavy rainfall, high bacterial levels have also been detected 2 days following flooding, implying that *E. coli* is capable of multiplying in bank soil, perhaps as a member of the natural flora (97). In summary, the studies described above support the notion that in the nutrient-rich tropics, *E. coli* is a member of the natural microflora (81).

SALMONELLA PASSAGE BETWEEN HOST SPECIES

Salmonella has been isolated from a large number of animal species (88). The majority of the 1.3 billion annual cases of *Salmonella*-caused human gastroenteritis result from ingestion of contaminated food products, such as undercooked beef, pork, chicken, seafood, and eggs (72). *Salmonella* infections are also contracted following consumption of fresh fruits or

vegetables that have been contaminated by infected fertilizer (100). Furthermore, as several reptile species are known *Salmonella* reservoirs (53, 54), reptilian pets have served as a source of *Salmonella* for infected owners (84). This indicates that *Salmonella* species lack special host adaptations and are capable of colonizing a wide variety of macroorganisms (32). The ubiquitous nature of *Salmonella* may facilitate a cyclic lifestyle consisting of passage through a host into the environment and back into a new host (104). Thus, unlike the commensal *E. coli*, which does not appear to survive in nonhost environments for extended periods of time in climatic regions other than the tropics, long-term survival of *Salmonella* in the secondary habitat ensures its passage to the next host (Fig. 1A).

The health and economic consequences associated with *Salmonella* contamination of food products have led to numerous studies of the survival and transmission of these bacteria in the poultry, pig, and cow industries (see references 41 and 106 for reviews). For example, *Salmonella* has been detected in several locations within cow farms, pig farms, and slaughterhouses, both before and after sacrifice (5, 48, 65); the same *Salmonella* clone has been recovered in a cow herd and in ground meat products following processing (65). Investigation of a piggery in Denmark revealed the presence of *Salmonella* in samples

from the animals, their environment, and their feed collected over a 2-year period (5). Again, all isolates originated from a single clone, suggesting that the same population persisted in the farmhouse environment. Waste slurry from infected pigs, which is used as an agricultural fertilizer, remained positive for culturable *Salmonella* up to 21 days after it was spread onto farmland outside the contaminated area (5). These studies indicate that *Salmonella* was continually introduced into the pig population through a cycle of animal shedding and short-term survival in the farm environment rather than from an external source (5). Long-term contamination of farms appears to be a widespread phenomenon as *Salmonella* persistence has been documented for more than 1 year in a poultry house, despite disinfections (24).

Birds and flies are important vectors for rapid, widespread dissemination of *Salmonella* in the environment (25). Infection of wild bird populations with salmonellae has been correlated with proximity to farms and/or the incidence of human salmonellosis in the same area (66). Likewise, adult muscoid flies (including the common housefly, *Musca domestica*) can carry *Salmonella*, particularly on dairy farms (infection rate, 67%) and poultry farms (infection rate, 13%). *Salmonella* survives in flies for up to 4 weeks, which is the life span of the flies (64). *Salmonella* has been observed to survive in infected blowfly pupae for 18 days at 5°C (52), despite physicochemical conditions that are dramatically different from those that exist in vertebrate hosts. Moreover, germfree adult houseflies inoculated with *Salmonella* show total defecation outputs of as much as 10⁷ CFU (37). Thus, flies that come in contact with contaminated materials (i.e., manure, food, and water) are capable of transmitting *Salmonella* (64).

Rapid dissemination of *Salmonella* between hosts also occurs by passage of bacteria from infected farm animals to vegetables as a result of field fertilization with raw, contaminated manure. Experimental inoculation of manure has revealed seasonal patterns of *Salmonella* survival in fertilized soil and transmission to crops (70). Detection of *Salmonella* on vegetables is correlated with warm, moist summer conditions (average daily temperature, >20°C), as no vegetable contamination was observed when manure was applied during the late fall (average daily temperature, <10°C) or following repeated freezing-thawing cycles (70). Contact of *Salmonella* with the flowers, stems, or fruits of tomato plants leads to infiltration and colonization of plant tissues (42, 43), and tomato plants grown hydroponically have been shown to take up *Salmonella* (44). In summary, the high nutrient content of sewage results in a good fertilizer, but the ability of *Salmonella* to survive in the fertilized soil may help perpetuate the presence of *Salmonella* species outside animal hosts (79).

Transmission of *Salmonella* between hosts is not limited to natural nonhost environments, as the presence of bacteria on household surfaces has important health consequences. A striking study of the survival of *Salmonella* in bathrooms and toilets following bacterium-induced illness demonstrated that there were high contamination levels for up to several weeks (7). Persistence was enhanced by adhesion and biofilm formation. Toilet seeding experiments designed to mimic conditions of acute diarrhea resulted in isolation of *Salmonella* from air samples immediately following flushing and in toilet bowl biofilm samples for up to 50 days (7). Inhalation has been found

to be a potential alternate route of *Salmonella* infection in pigs, given their rooting behavior (30). The presence of aerosolized salmonellae in bathrooms raises the possibility that inhalation is an alternative route of human infection.

SALMONELLA SURVIVES IN WATER AND SOIL

Salmonella is frequently isolated from water sources (21), which serve as bacterial reservoirs and may aid transmission between hosts (32). Like *E. coli*, *Salmonella* is constantly released into the environment from infected humans, farm animals, pets, and wildlife (8). Despite efforts to contain and sanitize human waste, *Salmonella* can survive for 10 to 15 days in a septic system (73), whereas *E. coli* has a negative growth rate in this environment (36). Seepage from septic tanks and sewage injection well fields, as well as sewage and storm runoff, facilitates bacterial passage into surface waters (8, 74). Detection of *Salmonella* correlates with proximity to the sewage discharge area (3, 8). For example, *Salmonella* was isolated from 65% of the water samples collected along the Peavine Creek in Decatur, Ga., which flows through urban industrial and residential areas (21). In addition, *Salmonella* was detected within 350 feet of the subsurface origin of a small stream in the same area, suggesting that contamination occurs rapidly once water emerges from the earth (21).

Compared to other bacteria, *Salmonella* has high survival rates in aquatic environments (20); it outlives both *Staphylococcus aureus* and the waterborne *Vibrio cholerae* in groundwater and in heavily eutrophied river water (27). The presence of *Salmonella* in marine environments does not vary seasonally and is independent of water temperature (3). Furthermore, *Salmonella* has a high survival rate following mixing of sewage effluent with brackish water, a transition that causes a dramatic increase in salinity (63), whereas osmotic stress has been implicated in the apparent death of *E. coli* in seawater (13). Thus, compared to *E. coli*, *Salmonella* appears to withstand a wider variety of stresses associated with environmental fluctuations and may persist in water environments for some time.

Salmonella can be widely disseminated in soil and sediment, even in the absence of active fertilization, as a result of water currents, underground springs, and rain runoff carrying contaminated material (1, 20). Percolation of wastewater through soil filters out bacteria that become trapped in this environment (20). *S. enterica* serovar Adelaide exhibited extended longevity in moist or saturated sand proximal to underground septic tanks (73). *Salmonella* has been detected frequently in environmental soil samples collected from both agricultural and recreational areas (1, 103–105). In contrast to *E. coli*, which has a low survival rate in soil (i.e., an average half-life of 3 days) (13, 101), *Salmonella* can survive and multiply for at least 1 year in this ecosystem (25, 104).

Soil and sediment particles are believed to function as microecological niches in which bacterial species can survive and perhaps replicate (14). Association with soil particles can provide bacteria with both high concentrations of nutrients, due to the release of organic molecules from attached algal cells, and protection against predation, by providing shelter against grazing protozoans (31). Adhesion of *Salmonella* cells to soil particles correlates with cell surface hydrophobicity (99), which is typically manifested by modification of the bacterial outer

membrane in response to changes in environmental conditions. The adhesive properties of *Salmonella*, as well as the nutrient-rich growth conditions provided by association with soil, may account for the fact that nearly 100% of the *Salmonella*-positive samples taken downstream of a sewage treatment plant over a 1-year period were samples obtained from the bottom sediment rather than samples obtained from the surface water (45). *Salmonella* has exhibited fourfold-greater adherence to some mineral particles than *E. coli* (99), although *E. coli* can survive in estuarine sediment environments (14). The ubiquitous nature and high rate of survival of *Salmonella* in soil and sediment may represent a critical adaptation to its cyclic lifestyle involving host and nonhost environments.

IS *E. COLI* A VALID INDICATOR SPECIES FOR *SALMONELLA*?

E. coli is typically used as the indicator species for environmental fecal contamination. However, this species serves as a relevant indicator only if its survival rate in a given environment is equal to or slightly higher than that of the bacterium of interest. Because the majority of nonhost environments appear to be dead ends for *E. coli* but a means to a new host for *Salmonella*, this premise may not always be valid. For example, *Salmonella* survives longer than *E. coli* in many nonhost environments, such as a brackish lagoon following discharge from a sewage treatment plant stabilization pond (63) and during exposure to polar aquatic conditions (93). Likewise, *Salmonella* was more resistant to killing by biotic factors (microbial predators or competing organisms) than *E. coli* in three drinking water sources in Sierra Leone (108), perhaps reflecting a difference in adhesion to protective particles. The rate of survival of *Salmonella* in estuarine water at temperatures below 10°C is significantly higher than that of *E. coli* (80). Furthermore, *Salmonella* grows better than *E. coli* on alfalfa sprouts, which are frequently irrigated; this may be due to the 1,000-fold-higher level of attachment of *Salmonella* to vegetation (6). In summary, the different rates of survival of *Salmonella* and *E. coli* in nonhost environments suggest that *E. coli* may not be an appropriate indicator of *Salmonella* contamination.

SPECIES-SPECIFIC GENES MEDIATING SURVIVAL IN NONHOST ENVIRONMENTS

A pairwise comparison of the *Salmonella* and *E. coli* genomes revealed that more than 1,100 *Salmonella* genes are absent from the *E. coli* genome and more than 800 *E. coli* genes are not present in the *Salmonella* genome (62). Furthermore, there are more than 1,000 genes in both genomes whose functions remain unknown and which could contribute to proliferation in nonhost environments, such as soil and water. While a comprehensive genomic analysis of these two organisms is beyond the scope of this paper, below we discuss a few examples of genes that may contribute to the different abilities of *Salmonella* and *E. coli* to survive in nonhost environments.

The majority of nonhost environments are characterized by thermal variability, high osmolarity, pH fluctuations, and low nutrient availability, suggesting that a stress response is activated in *Salmonella* and *E. coli* in such environments. One regulatory protein involved in such a response is the stationary-

TABLE 1. *Salmonella*-specific genes not implicated in virulence

Gene	Putative function	Reference(s)
<i>cob</i>	Vitamin B ₁₂ biosynthesis	39, 62
<i>fljA</i>	Repressor of phase 1 flagellin gene	39, 62
<i>fljB</i>	Flagellar subunit	39, 62
<i>iicA</i>	Unknown	75
<i>iviXXII</i>	Unknown	23
<i>iviXVII</i>	Unknown	23
<i>iviXIX</i>	Unknown	23
<i>mgtB</i>	Magnesium transport	96
<i>nanH</i>	Sialidase	39, 62
<i>pcgL</i>	D-Ala-D-Ala dipeptidase	46, 68
<i>phoN</i>	Phosphatase	40, 51
<i>sinR</i>	Transcriptional regulator	39, 62

phase alternative sigma factor RpoS (28, 56), which is expressed when *Salmonella* and *E. coli* are present in seawater (69). There was a 1,000-fold-greater decrease in the number of culturable cells for an *E. coli* strain lacking the *rpoS* gene than for the isogenic wild-type strain following 8 days of exposure to seawater (69). In contrast, inactivation of the *rpoS* gene decreased the number of culturable *Salmonella* cells only 10-fold (69), suggesting that *Salmonella* may employ additional genetic mechanisms to respond to stresses in this environment.

Salmonella harbors several genes that, although absent from related enteric species, are not required for virulence, suggesting that they may be involved in survival in nonhost environments (Table 1). These genes encode products involved in nutrient acquisition and utilization, motility, and transcriptional regulation, as well as genes having unknown functions. For example, *Salmonella* has a periplasmic D-Ala-D-Ala dipeptidase, termed PcgL, that allows growth on D-Ala-D-Ala (46), which originates during peptidoglycan remodeling and is also released from the cell walls of dead bacteria. Inactivation of the *pcgL* gene renders *Salmonella* hypervirulent in mice but defective for survival in nutrient-poor conditions (68), such as those that it might encounter outside an animal host. This has led to the hypothesis that despite the virulence-attenuating effects of the *pcgL* gene, *Salmonella* has retained this gene because of the contribution that it makes to fitness in nonhost environments (68).

The *E. coli* genome also encodes a D-Ala-D-Ala dipeptidase, termed DdpX, that localizes to the cytoplasm (58). Although the PcgL and DdpX proteins exhibit a low level of amino acid identity (40%), they have almost identical enzymatic activities (46, 58). However, these proteins differ in their sites of action; the *Salmonella* D-Ala-D-Ala dipeptidase encounters its substrate in the periplasm (46), whereas the *E. coli* enzyme encounters its substrate in the cytoplasm following uptake by a D-Ala-D-Ala transporter encoded in the same operon as the *ddpX* gene (58). This suggests that *Salmonella* and *E. coli* evolved different solutions to the same problem: the utilization of peptidoglycan-derived fragments in nutrient-limited environments (46, 58, 68). Transcription of the *Salmonella pcgL* gene is regulated by the PhoP-PhoQ two-component system, which is activated in response to low-magnesium conditions (98), and does not require the RpoS protein. On the other hand, expression of the *E. coli ddpX* gene is regulated by RpoS under nutrient-rich conditions (58) and by the NtrC-NtrB two-component system in nitrogen-poor environments (109). The

differences in regulation of the *Salmonella* and *E. coli* D-Ala-D-Ala dipeptidases imply that these enzymes are used in different environments.

The *phoN* gene of *Salmonella* encodes a periplasmic non-specific acid phosphatase (40, 51) that exhibits sequence similarity to an acid phosphatase from *Citrobacter* sp. strain N14, which has been implicated in detoxification of heavy metals that accumulate in metal-contaminated soil (67). *E. coli* lacks the *phoN* gene but contains a periplasmic alkaline phosphatase (termed PhoA), which is absent from *Salmonella* (62). Like the D-Ala-D-Ala dipeptidases described above, the phosphatases of *E. coli* and *Salmonella* are regulated differently: the *E. coli phoA* gene is transcriptionally induced under phosphate starvation conditions by the PhoB-PhoR regulatory system (107), whereas the *Salmonella phoN* gene is transcriptionally regulated by the PhoP-PhoQ system (38, 51). Together with the different pH optima, this suggests that *Salmonella* and *E. coli* use their periplasmic phosphatases in different milieus.

Mg²⁺ is the most abundant divalent cation in living organisms, and bacteria are equipped with systems that sense and transport Mg²⁺ (94). Both *Salmonella* and *E. coli* contain the constitutive Mg²⁺ transporter CorA (50), the low-Mg²⁺-induced Mg²⁺ transporter MgtA (11, 62, 95), and the Mg²⁺-responding PhoP-PhoQ two-component regulatory system (38). On the other hand, *Salmonella*, but not *E. coli*, contains the *mgtCB* operon, which encodes a third Mg²⁺ transporter termed MgtB (95, 96) and the MgtC membrane protein, which is required for normal growth under Mg²⁺-limiting conditions (10). *Salmonella* can grow in the presence of lower Mg²⁺ concentrations than *E. coli*, and introduction of the *mgtC* gene is sufficient to enhance *E. coli* growth under Mg²⁺-limiting conditions (10). In contrast to the *mgtC* gene, the *mgtB* gene is not required for virulence, suggesting that it may enhance *Salmonella* survival in nonhost environments.

CONCLUSIONS

E. coli populations appear to exist in two distinct states outside the host. On the one hand, *E. coli* cells that are shed from a mammalian host find themselves at a dead end in the external environment because of their inability to survive environmental biotic and abiotic antagonists and because of the low probability of replacing the resident strain of another host (33, 34, 86, 89, 90). On the other hand, *E. coli* is a member of the normal flora in certain tropical ecosystems, possibly due to the high nutrient concentrations, constant temperatures, and moist conditions of the environments (49), which mimic the mammalian colon. At this time it is not known how similar the *E. coli* strains that live in our guts are to the strains that live in tropical ecosystems.

One way in which *Salmonella* differs from *E. coli* is that its enhanced survival in the external environment promotes transmission to a new host. Detection of *Salmonella* in the environment correlates with human or animal activity. Because *Salmonella* is able to infect a large number of animal species, including flies, identification of the source of environmental contamination may not always be possible. Nevertheless, survival of *Salmonella* in the soil, in water, and on a variety of surfaces provides the bacterium with an increased probability of infecting a new host.

The differences between *Salmonella* and *E. coli* in terms of survival outside animal hosts result from genes that are specific to each of these species. However, other genetic mechanisms, such as disparate regulation and/or allelic differences between homologous genes, may also contribute to the distinct lifestyles of these enteric microorganisms.

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