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Prevalence of Surface Swarming Behavior in *Salmonella*

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Swarming behavior among 167 *Salmonella* sp. isolates, representing all eight groups, was assessed. Only eight strains failed to swarm under standard conditions. Four of the defective strains swarmed on alternate carbon sources, and four harbored general defects in motility or lipopolysaccharide. Thus, swarming may represent an evolutionarily conserved behavior in *Salmonella* spp.

Swarming behavior has been traditionally described as a bacterial surface motility phenotype observed on laboratory media (10). Some of the earliest descriptions include those by Hauser (15) in 1885 for *Proteus* spp. and by Gard (11) in 1938 for *Salmonella* spp. A series of reports between 1970 and 1980 described the potential utilization of the swarming behavior as a means for detecting and isolating *Salmonella* spp. from human fecal samples (21–23, 26, 27). However, a comprehensive description of the swarming behavior in both *S. enterica* serovar Typhimurium and *Escherichia coli* was unavailable until 1994 (14). When propagated on glucose-supplemented nutrient-rich semisolid medium, serovar Typhimurium undergoes a morphological differentiation into swarmer cells, rendering them physically capable of active surface migration (14). In direct comparisons to the vegetative swimmer population (i.e., propagated in broth with identical nutrient composition), swarmer cells are generally longer and hyperflagellated (14) but not hyperflagellated to the same extent as that observed in *Proteus mirabilis* (4).

Virulence attributes are also coregulated with differentiation in several pathogenic organisms, implicating physiological relevance of the swarming behavior beyond a laboratory-driven motility phenotype. These include hemolysin and protease in *P. mirabilis* (2, 32) and degradative enzymes (e.g., those involved in breakdown of mucin) or toxins in *Serratia liquefaciens*, *Clostridium septicum*, and *Bacillus cereus* (12, 24, 30). Type III secretion systems and the flagellar apparatus are thought to be evolutionarily related (25), and virulence factors have been observed to be secreted through the flagellar export apparatus in *Yersinia enterocolitica* (34).

We have also described several important physiological attributes that are coregulated with swarmer differentiation in serovar Typhimurium. Swarmer differentiation is coupled to elevated resistance to a wide variety of structurally and functionally distinct classes of antibiotics (17). One mechanism of resistance was directly attributed to the up-regulation of genes (*pmr*) that confer resistance to cationic peptides (19), important for survival in the murine gastrointestinal environment (13). Proteomic analyses revealed that differentiation results in a global shift in metabolism from catabolism to anabolism, including reduced outer membrane permeability coupled with activation of de novo biosynthetic pathways (18). Accordingly, the general nutrient-rich requirements for initiating swarmer differentiation were redundant for maintaining the differentiated cells in the swarm state (18). Given that there is little to no information in the literature regarding the prevalence of swarming behavior within the genus, we assessed swarming behavior among 167 *Salmonella* isolates, representing *S. bongori* and all seven subspecies of *S. enterica*.

Salmonella reference collections B (SARB) and C (SARC) were screened for swarming behavior. SARB represents 37 distinct serovars in *S. enterica* subspecies group I (6), and SARC is composed of 96 strains, representing all seven subspecies groups of *S. enterica* and *S. bongori* (designated group V) (7). All strains were screened for both swimming (NBG [Difco nutrient broth, 0.5% glucose] with 0.25% Difco agar) and swarming (NBG with 0.5% agar) motility as previously described (19). All SARB strains exhibited swimming motility, but four strains failed to swarm (Table 1). The four nonswarmers did not belong to a particular serovar and represented the minority, since all others within the respective group were swarm proficient. One exception was serovar Senftenberg (SARB59), which was represented by only one strain. Given the comprehensive nature of SARB, it may be concluded that swarming is a universally shared behavior among the strains in the *S. enterica* subspecies group I. Similarly, only four strains from SARC failed to swarm, but two of those strains also failed to exhibit swimming motility (SARC9 and SARC26) (Table 2). Thus, the inability of SARC9 and SARC26 to swarm is likely due to defects in the flagellar apparatus or motor. It should be also noted that SARC9 and SARB63 (which is swim and swarm proficient) are supposed to represent the same strain (7). The observed discrepancy in the motility behaviors of the two strains may be due to the acquisition of a mutation in SARC9 with some respect to flagellar function (K. Sanderson, personal communication).

To date, only lipopolysaccharide (LPS)- and flagellum-related mutations have been shown to unconditionally abolish the swarming behavior in serovar Typhimurium. However, the LPS-associated swarm defect is only at the level of motility, since all mutants retained their abilities to fully differentiate into swarmers, and surface motility could be partially restored by exogenous supplementation of surfactants (31). LPS and

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TABLE 1. Summary of motility behaviors of *Salmonella enterica* subspecies group I strains (SARB)[a]

Serovar	No. of strains tested	No. of strains with indicated motility[b]	
		Swim	Swarm
Agona	1	1	1
Anatum	1	1	1
Brandenburg	1	1	1
Choleraesuis	4	4[c]	3[d]
Decatur	1	1	1
Derby	3	3[c]	3
Dublin	3	3	3
Duisburg	1	1	1
Enteritidis	4	4	4
Emek	1	1	1
Gallinarum	1	1	1
Haifa	1	1	1
Heidelberg	2	2	2
Indiana	1	1	1
Infantis	2	2	2
Miami	2	2	2
Montevideo	2	2	2
Muenchen	4	4	3[d]
Newport	3	3	3
Panama	3	3	3
Paratyphi A	1	1	1
Paratyphi B	5	5	5
Paratyphi C	3	3	2[d]
Pullorum	2	2	2
Reading	1	1	1
Rubislaw	1	1	1
Saintpaul	2	2	2
Schwarzengrund	1	1	1
Sendai	1	1	1
Senftenberg	1	1	0[d]
Stanley	1	1	1
Stanleyville	1	1	1
Thompson	1	1	1
Typhi	2	2	2
Typhimurium	4	4	4
Typhisuis	2	2	2
Wien	2	2	2

[a] The basal medium is nutrient broth (0.5% glucose [wt/vol]) solidified with 0.5% (swarm) or 0.25% (swim) agar. All motility assays were performed at least three times to ensure reproducibility.

[b] The entire plate was colonized after 24-h incubation at 37°C (SARB) or as noted otherwise. A strain was declared to be nonmotile in the absence of visible colony migration.

[c] SARB5 (serovar Choleraesuis) and SARB9 (serovar Derby) did not reach the edges of the plates but greater than half the plates were colonized.

[d] Swarm-deficient strains: SARB5, SARB32 (serovar Muenchen), SARB48 (serovar Paratyphi C), and SARB59 (serovar Senftenberg).

TABLE 2. Summary of motility behaviors of *S. bongori* and *S. enterica* strains (SARC)[a]

Subspecies (species)	No. of strains tested	No. of strains with indicated motility[b]	
		Swim	Swarm
I	11	10[c]	10[d]
II	22	21[c]	21[d]
IIIa	4	4	4
IIIb	5	5	5
IV	28	28[e]	27[d]
V (*S. bongori*)	12[f]	12	11[d]
VI	9	9	9
VII	4	4	4

[a] The basal medium is nutrient broth (0.5% glucose [wt/vol]) solidified with 0.5% (swarm) or 0.25% (swim) agar. All motility assays were performed at least three times to ensure reproducibility.

[b] Entire plate was colonized after 24-h incubation at room temperature and 37°C or as noted otherwise. A strain was declared to be nonmotile in the absence of visible colony migration.

[c] Swim-deficient strains were SARC9 (subspecies I) and SARC26 (subspecies II).

[d] Swarm-deficient strains were SARC9, SARC26, SARC67 (subspecies IV), and SARC76 (subspecies V [*S. bongori*]).

[e] SARC59 did not reach the edges of the plates, but more than half the plates were colonized.

[f] No data were recorded for SARC71 because it failed to grow regardless of medium or temperature.

extracellular polysaccharides (EPS) may function as general surfactants to promote surface migration. EPS has a general protective role as an antidesiccant, since it can absorb large amounts of water (28). O antigen is also a component of the EPS, as it can slough off from the cell surface (8). The physicochemical characteristics of LPS and EPS likely provide a hydrated shell around the swarming colony, enabling rotation of flagella for surface movement. Accordingly, LPS biosynthetic pathways have been shown to be up-regulated during the initial stages of swarmer differentiation (33). Thus, SARB and SARC strains exhibiting the swim-proficient/swarm-defective phenotype may harbor physical defects in their LPS compositions. To test this hypothesis, LPS from all swarm-defective and several swarm-positive strains was prepared as described by Hitchcock and Brown (16), and the profiles were subsequently visualized by silver staining (9). Indeed, as summarized in Table 3, two of the four swarm-defective SARB strains exhibited a rough LPS profile, indicative of lacking the O-antigen component. SARB5 was previously reported to be a rough strain, but there is no record of such for SARB33 (6). Although the remaining swarm-defective strains apparently possess intact LPS, mutations in LPS modification genes can also abolish swarming in serovar Typhimurium (17, 31).

Even under nutrient-rich conditions, supplementation of an energy-rich carbon source, such as glucose, is essential for stimulating active swarming in serovar Typhimurium (14, 18). Accordingly, mutations in the phosphotransferase system (PTS) abolish swarming in serovar Typhimurium, but swarming is restored when supplemented with non-PTS sugars such as *N*-acetylglucosamine or arabinose (14). To determine whether the swarm-defective phenotype in the SARB and SARC strains could be conditionally rescued, swarming was reassessed in the presence of PTS or non-PTS carbon sources other than glucose (i.e., nutrient broth with 0.5% carbon source and 0.5% agar). As summarized in Table 3, the two rough strains (SARB5 and SARB33) and the two swim-defective strains (SARC9 and SARC26) failed to swarm regardless of alternate carbon sources. In contrast, serovar Typhimurium ATCC 14028 (14028) and two randomly chosen swarm-proficient strains from the initial screens (SARB9 and SARC59) maintained their ability to swarm under all conditions. As represented by those from 14028, distinct swarm patterns were observed in the presence of different carbon sources, but the PTS sugars generally produced a similar pattern (Fig. 1). These phenotypic differences may be the result of sugar-specific changes in the production of LPS and extracellular slime, differentially affecting the general physicochemical properties of the surface in wetness and surfactants. With the exception of SARC76 (*S. bongori*), which poorly swarmed only in the pres-

TABLE 3. Summary of swarming in the presence of different carbon sources by serovar Typhimurium 14028 and various SARB and SARC strains[a]

Strain	Colonization on agar with indicated carbon source												Presence of phenotype	
	PTS					Non-PTS								
	Glu	Fru	Gal	Man	Sor	Ara	Gly	Mal	Raf	Succ	Suc	Xyl	Swim[c]	Oag[d]
SARB5	−	−	−	−	−	−	−	−	−	−	−	−	+	−
SARB33	−	−	−	−	−	−	−	−	−	−	−	−	+	−
SARB48	−	+	++	++	+	++	−[b]	+	+	−[b]	−[b]	+	+	+
SARB59	−	−	−	−	+	−	++	+	−[b]	−[b]	−[b]	+	+	+
SARC9	−	−	−	−	−	−	−	−	−	−	−	−	−	+
SARC26	−	−	−	−	−	−	−	−	−	−	−	−	−	+
SARC67	−	++	++	++	++	++	+	++	−[b]	−	−	++	+	+
SARC76	−	−	−	+	−	−	−	−	−	−	−	−	+	+
14028	++	++	++	++	++	++	++	++	++	++	++	++	+	+
SARB9	++	++	++	++	++	++	++	++	++	++	++	++	+	+
SARC59	++	++	++	++	++	++	++	++	++	++	++	+	+	+

[a] The basal medium is nutrient broth (0.5% agar) supplemented with different carbon sources (pH adjusted to 7 when necessary). Ara, arabinose; Fru, fructose; Gal, galactose; Glu, glucose; Gly, glycerol; Man, mannose; Mal, maltose; Raf, raffinose; Sor, sorbitol; Succ, succinate; Suc, sucrose; Xyl, xylose. SARC76 is the only *S. bongori* strain; all others are *S. enterica.* 14028, wild-type strain of serovar Typhimurium (ATCC 14028). Two plus signs denote that the entire surface of the swarm plate was colonized, one plus sign denotes that greater than half the surface was colonized, and a minus sign denotes that there was no swarming (i.e., no visible movement away from the site of inoculation). All observations were made after 24-h incubation at 37°C, and all assays were performed at least three times to ensure reproducibility.

[b] The colony grew slightly beyond the site of inoculation, but no active swarming was observed.

[c] Regarding swimming on NBG (0.25% agar), cells were either motile (+) or nonmotile (−).

[d] A plus sign or a minus sign denotes the presence or absence of the O antigen (Oag) component of LPS as determined by silver staining.

ence of mannose, the remaining three swim-proficient/swarm-defective mutants from the initial screens were conditionally rescued by various carbon sources. SARB48 and SARC67 were able to swarm when supplemented with PTS sugars other than glucose, but several non-PTS carbon sources also restored swarming. In contrast, sorbitol was the only PTS sugar that promoted swarming in SARB59, which exhibited swarming comparable to that of 14028 only in the presence of glycerol.

Despite the fact that members of the genus *Salmonella* are closely related genetically, tremendous variations exist in host targets, virulence, and disease manifestations. It is estimated that all salmonellae shared a common ancestor 25 million to 40 million years ago (29). Both acquisition (via phages and horizontal transfer) and loss (via point mutation, insertional inactivation, and deletion) of functional genes played important roles throughout the development of host specificity among the different serovars of *Salmonella* (3). Thus, conservation of common phenotypes within the entire genus may be indicative of their important role in survival and persistence within or outside the general host environment. This study provides some convincing evidence that swarming may be one of these conserved phenotypes. With the exception of the four strains that were defective in either flagellar function or LPS structure, 97.5% (159/163) of the strains representing the entire genus exhibited the swarming behavior in the presence of glucose, and 100% (163) proved to be swarm proficient when supplemented with an alternate carbon source. The majority of salmonellae favor a gastrointestinal lifestyle regardless of the specific host(s), with minor exceptions including those that have adapted to the environment within the urinary tract (1). The in vitro growth conditions that permit swarming may be in some aspects physiologically relevant to the nutrient-rich environment of the gastrointestinal tract (5). Although the swarming behavior is not limited to 37°C (14), the observed rich-nutrient requirements may present a rather large obstacle for this behavior to exist outside the host. The gastrointestinal tract is considered to be a nutrient-rich environment (5), and serovar Typhimurium can swarm in the presence of significant levels of bile (W. Kim and M. G. Surette, unpublished results). A previous study also established that swarming occurs under strictly anaerobic conditions, and the disruption of the *shdA* gene, which contributes to the persistence of *Salmonella* in the intestine at the level of fibronectin binding (20), also conditionally abolishes the swarming behavior (31). In addition to the motility phenotype, swarm cells of serovar Typhimurium undergo a systemic metabolic differentiation characterized by reduced expression of proteins involved in the uptake of exogenous nutrients and outer membrane permeability (18). This

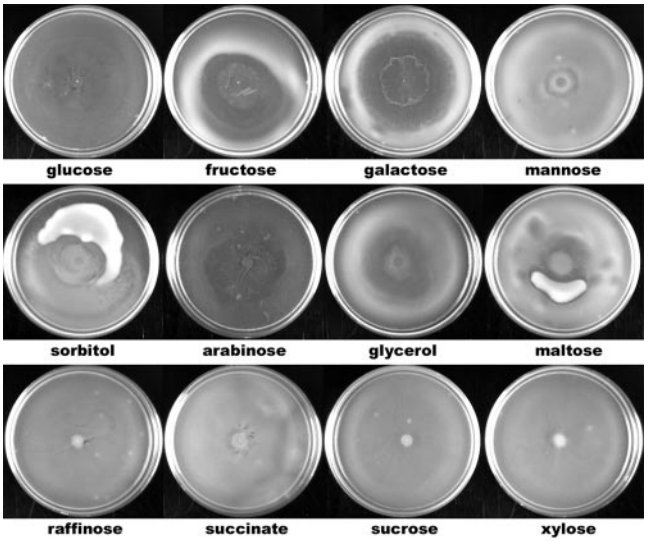

FIG. 1. Swarming behavior of serovar Typhimurium in the presence of various carbon sources. Swarm medium is nutrient broth (0.5% agar) supplemented with the indicated carbon sources (0.5% [wt/vol]). All images were captured after 24 h at 37°C.

may represent a sophisticated survival strategy within the gastrointestinal environment. The potential trade-off between reduced utilization of exogenous nutrients and resistance to antimicrobial mechanisms of the host and indigenous microflora may confer an ecological advantage to *Salmonella*. In fact, laboratory conditions that trigger swarmer differentiation in *Salmonella* may fortuitously stimulate the cells to enter a robust physiological state relevant to the lifestyle in the gastrointestinal environment. Based on these observations and the data presented here, we propose that prevalence of the swarming behavior among the serovars is a measure of its evolutionary importance, adapted to the gastrointestinal environment.

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