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Full Length Research Paper

Interaction of *Salmonella typhimurium* and *Salmonella enteritidis* with polystyrene does not correlate with virulence in young chickens

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***Salmonella typhimurium* and *Salmonella enteritidis* are the most frequently isolated serotypes in human and animal Salmonella infections. The *in vitro* surface colonization characteristics of *S. typhimurium* L1388 and *S. enteritidis* L1225 on hydrophobic surfaces were assessed with a view to understanding their surface preference in relation to *in vivo* virulence. Although both *S. typhimurium* and *S. enteritidis* preferentially colonized polystyrene under normal nutrient-rich growth medium, *S. typhimurium* formed significantly (P < 0.05: P = 0.000008) smaller amounts of biofilm than *S. enteritidis*. The biofilm formed on polystyrene was optimum at different times, 200 min and 400 min for *S. enteritidis* and *S. typhimurium*, respectively. *S. typhimurium* also formed significantly (P < 0.05) less biofilm than *S. enteritidis* when the growth medium was supplemented with 100 mM each of either D-(+)-mannose (P = 0.0001), D-(+)-glucose (P = 0.0005), D-(-)-mannitol (P = 0.00002) or xylose (P = 00009). Biofilms formed by *S. enteritidis* following growth in sugar-supplemented medium were not significantly different from that following growth in non-supplemented medium; but significant (P < 0.05) reduction in amounts of biofilm formed by *S. typhimurium* were produced by only mannitol (P = 0.0008) and xylose (P = 0.00004). Growth in sodium chloride-supplemented medium resulted in significantly (P < 0.05) less biofilm formed by both *S. typhimurium* (P = 0.0084) and *S. enteritidis* (P = 0.0002); even though the quantity formed by *S. typhimurium* was significantly (P < 0.05: P = 0.0098) more than that by *S. enteritidis*. Both strains formed significantly less biofilm on polystyrene when cultured in a starvation medium for 24 h. They also do not differ significantly from each other in their extent of adherence to polystyrene and 14-day chick mortality. Overall, the interaction of *S. typhimurium* and *S. enteritidis* with polystyrene does not correlate with virulence in young chickens.**

Key words: Biofilm, *Salmonella typhimurium*, *Salmonella enteritidis*, chickens, surface.

INTRODUCTION

Salmonella enterica is a facultative intracellular pathogen capable of causing disease in a wide range of host species. The non-typhoid *S. enterica* are among the common causes of bacterial gastroenteritis (Fierer and Swancutt, 2000); and the serotypes Typhimurium and Enteritidis are more frequently isolated in human, swine, avian and bovine salmonellosis (Schwartz, 1999). Thus, they are a public health problem worldwide, particularly with respect to food safety (Lax et al., 1995) as contaminated food of animal origin, particularly poultry, remains the major source of these pathogens (D`Aoust, 1994; Guard-Peter, 2001). With the exception of very young chicks, Enteritidis and Typhimurium rarely cause clinical disease, but can colonize the gut of poultry

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λThe study was done during a visit to Animal Health Laboratory, School of Agriculture, Ibaraki University, Ami-machi, Ibaraki, Japan.

Table 1. Sensitivity[1] of test strains to common antibiotics.

Strains	Source	Country of isolation	Minimum inhibitory concentration (MIC) in µg ml^{-1}										Resistance
			A	T	C	F	G	K	S	Su	TMP	NA	
ST	Cattle	Japan	1	2	8	8	0.5	2	32	64	0.5	4	SSu
SE	Cattle	Japan	1	2	8	8	0.5	1	8	64	1	4	SSu

ST, *S. typhimurium* L1388; SE, *S. enteritidis* L1225: A, ampicillin; T, tetracycline; C, chloramphenicol; F, florfenicol; G, gentamicin; K, kanamycin; S, streptomycin; Su, sulfamethoxazole; TMP, trimethoprim; and NA, nalidixic acid. [1]Sensitivity expressed as MIC in µg ml^{-1} was determined by the method of National Committee of Clinical Laboratory Sciences (NCCLS) of the United States of America. Briefly, two-fold (doubling) serial dilutions of each antimicrobial agent from 0.004 to 512 µg ml^{-1} were prepared in Mueller-Hinton Agar. Overnight Brain Heart Infusion cultures of bacteria were diluted 1: 100 with normal saline, and the plates were spot-inoculated with 5 µl of the diluted cultures (containing approximately 1x 10^5 cfu of bacteria). The inoculated plates were incubated for 18-24 h at 37°C, and the minimum concentration of the antimicrobial agents that inhibited growth of bacteria was recorded.

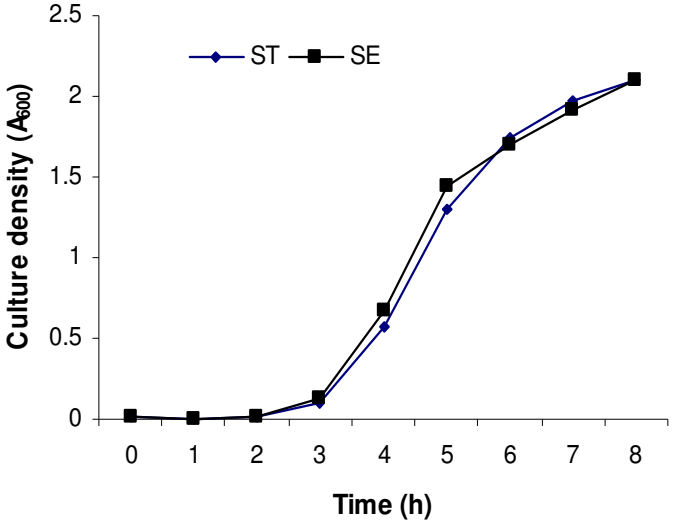

Figure 1. Baseline growth of test strains. Bacteria were grown in Luria-Bertani (LB) broth and growths monitored by absorbance (A) measurements at 600 nm of bacterial culture during incubation with shaking at 37°C. ST, *S. typhimurium* L1388; SE, *S. enteritidis* L1225.

(Barrow et al., 1987; Barrow and Lovell, 1990). Enteritidis may also colonize the reproductive tract, leading to the contamination of eggs (Humphrey, 1999).

Bacteria exist in natural, clinical and industrial settings in form of sessile, highly structured community called 'Biofilm', defined as matrix-enclosed bacterial population adherent to each other and/or to surfaces or interfaces (Costerton et al., 1995; Potera, 1996). Although the formation of biofilm by bacteria is a survival strategy against environmental stress (Bradshaw, 1997), colonization of mucosal surfaces by pathogenic bacteria is also believed to proceed via biofilm formation (Costerton et al., 1987), and has been correlated with *in vivo* virulence in *S. enteritidis* (Solano et al., 1998; Bonafonte et al., 2000). Thus, biofilms represent a source of persisting and relapsing infections, and as a result, contribute significantly to pathogenesis (Costerton et al., 1987; Costerton et al., 1999).

Although the ability of *S. enterica* to form biofilm on diverse surfaces has already been reported (Joseph et al., 2001; Mirelles II et al., 2001; Somers et al., 1994), serotype- and surface-dependent differences, as well as the influence of culture conditions on biofilm formation have not been elucidated. The primary objective of our study is the comparison of biofilm formation by *S. typhimurium* and *S. enteritidis* with the ultimate aim of understanding the colonization process of these serotypes. Given the impact of biofilm, it is important to understand how these serotypes colonize their environment and the factors that influence the process. This is needed to understand how they cause disease and the colonization processes both in terms of animal and public health.

MATERIALS AND METHODS

Bacterial isolates and culture media

S. typhimurium L1388 and *S. enteritidis* L1225 were used in this study. Relevant characteristics of the isolates are as shown in Table 1 and Figure 1. The strains were propagated in either trypticase soy broth (TSB; BBL, U.S.A.), Luria-Bertani broth (LB; Daigo, Inc., Japan) or as indicated in the text; solid media cultures were grown on trypticase soy agar (TSA; BBL, U.S.A.). Biofilm formation was assessed in the following media: LB, starvation medium (ATM: 60 mM NaCl, 30 mM $NaHCO_3$, 20 mM KCl, 111 mM D-glucose, distilled water to 1000 ml; Gamazo et al., 1997), LB supplemented with 10% NaCl (LB-S), LB supplemented with 100 mM of either of D- (+)-glucose (LB-G), D- (+)-mannose (LB-M), D- (-)-mannitol (LB-ML), and xylose (LB-XY). Unless otherwise indicated, all chemicals used were purchased from Wako Chemical Company, Japan.

Biofilm formation assay

The microtitre plate assay used in this study was adapted from O`Toole and Kolter (1998). Briefly, bacteria were grown in LB broth overnight at 37 °C and 10 µl was inoculated into either of 1.5-ml microfuge tube (polypropylene), flat-bottom 24-well polystyrene tissue culture plate (FALCON^R; Becton Dickinson & Co., U.S.A.), and 96-well polyvinyl chloride microtiter plate containing 90 µl of LB broth. Plates were incubated at 37 °C for 24 h. Cultures were then removed, rinsed three times with sterile distilled water to remove unattached bacteria. The tubes or wells were either air-dried at

room temperature or dried at 37 °C, and adherent bacteria were stained at room temperature with 200 µl of 1 % w/v aqueous solution of crystal violet (Merck, Germany) for 20 min. The tubes or wells were rinsed three times with sterile distilled water after the dye was removed, and dried as before; stained adherent cells were detached from the walls of the tubes or plates using 300 µl of dimethylsulfoxide (DMSO; Merck, Germany), and the absorbance at 600 nm (A600), of the solubilized biofilm extracts were measured using a UV-2200 Spectrophotometer (Shimadzu Corporation, Japan). Results are means of at least three experiments.

Time-course assay of biofilm formation on polystyrene

Cultures of the isolates were grown in LB broth in polystyrene tissue culture plate over a period of 15 h at 37 °C, during which biofilm was quantified by the same method as described above. Results are means of a single representative triplicate determination.

Scanning electron microscopy

Ten milliliter LB broth containing polystyrene chips (0.5 cm x 0.5 cm; cut from plates made of this material and pre-sterilized with 100% ethanol) were inoculated with one colony of a particular isolate, and incubated at 37 °C without shaking for 24 h. The chips were then removed, washed three times with 50 mM sodium phosphate buffer (pH 7.0) and fixed by immersion in 3% glutaraldehyde-50 mM sodium phosphate buffer (pH 7.0) for 2 h on ice. Subsequently, the chips were washed three times with buffer, post-fixed by immersion in 1% osmium tetroxide-50 mM sodium phosphate buffer (pH 7.0) for 1 h at room temperature, washed three times with buffer, and dehydrated through graded ethanol-water mixtures in the following order: 25% (15 min), 50% (15 min), 75% (15 min), 90% (15 min) and 100% (15 min x 3). Dried chips were then immersed in 100% ethanol: ter-butyl alcohol (1:1) for 10 min twice, transferred to ter-butyl alcohol for another 10 min, later to 1 ml (just sufficient to cover the chip) of ter-butyl alcohol and kept at -20 °C to freeze for 1 h. Frozen samples were then vacuum-dried for 24 h in a vacuum freeze dryer (Eiko Engineering Co. Ltd., Japan) at 15 lb in-2 pressure, mounted on SEM sample tub using double stick tape, sputter-coated with osmium gas discharging at 1.1-1.2 kV voltage and 2-4 mA current using JOS-100D Osmium Plasma Coater (Jeol Technics Co. Ltd., Japan) at 0.9 Pa pressure and viewed with a JSM-T300 Scanning Electron Microscope (Jeol Technics Co. Ltd., Japan) at a constant accelerating voltage of 10 kV. Photographs were taken on Fuji Polaroid film. Two repeat experiments were carried out to confirm our observations.

Effect of carbon sources on biofilm formation

Bacteria were grown in LB, LB-G, LB-M, LB-ML, or LB-XY broths, and biofilm formed in polystyrene tissue culture or PVC microtitre plates was quantified as described earlier. Results are means of at least three experiments. Our choice of 100 mM as test sugar concentration was arbitrary, and based on previous works in which glucose at concentration up to 111 mM either did not affect biofilm formation or enhanced it in *E. coli* and *S. enteritidis* (Pratt and Kolter, 1998; Bonafonte et al., 2000).

Effects of starvation and osmotic stresses on biofilm formation

Biofilm formation was quantified after 24-h culture of bacteria in starvation medium (ATM broth; Gamazo et al., 1997) and high osmotic (salt) stress (LB-S medium) in 24-well polystyrene tissue culture plate using the same procedure as described earlier. Results are means of at least three independent determinations.

Adherence assay

The *in vitro* adherence of the isolates to polystyrene was assayed based on Onaolapo et al. (1997). Briefly, each isolate was grown with shaking (65 rpm) for 24 h in 10 ml LB broth containing two pieces (1 cm x 1 cm) of polystyrene cut from culture plate made of this material (the cut pieces were pre-sterilized by socking in 100% ethanol for 1 h and drying aseptically). The polystyrene chips were then removed, washed through two 10-ml rinses of normal saline (0.9 g NaCl, Distilled water to 100 ml), and each piece transferred into separate 9.9 ml normal saline (this gives approx. 1:100 dilution), vortex-mixed vigorously for 2 min, and plated out on TSA after appropriate dilution in normal saline. Plates were incubated at 37°C for 24 h, and colonies were counted. Results are means of at least three independent determinations.

Mortality and oral median lethal dose (LD_{50}) in chick model

Day-old white leghorn chicks (average body weight: 33.91 ± 3.32 g) were maintained in isolators in a previously fumigated room. The birds were starved of food and water for six hours prior to infection with test strains, but received food and water afterwards. The chickens were randomized into groups of ten birds and were infected orally (by delivering the doses directly into their mouth and allowing them to suck from the micropipette tip) with 5 x 10^2, 5 x 10^4 or 5 x 10^6 cfu of the corresponding test strain in 200 µl of PBS (pH 7.2). The infective doses were estimated retrospectively by plating appropriate dilution of a 24-h, 37°C-grown brain heart infusion broth-culture in PBS (pH 7.2). The control group, which consisted of 15 birds, received PBS only. The number of dead birds was recorded two times daily at twelve hours interval for 21 days, and the 50% lethal dose (LD_{50}) of each strain was calculated at day 14 post-infection by the method of Reed and Muench (1938).

Statistical Analysis

Data were analyzed by the one-way analysis of variance (ANOVA) using WebStat 2.0 (West, W.R. and Ogden, T.R. October 6, 2000, posting date. [Online] Department of Statistics, University of South Carolina, Columbia, U. S. A. http://www. stat.sc.edu/webstat/; June 20, 2002 [date last accessed]), and significance of results determined at the 5% probability level.

RESULTS

Biofilm formation

S. enteritidis formed significantly ($P < 0.05$) more biofilm than *S. typhimurium* in the nutrient-rich LB medium ($P = 0.000008$) after 24 h of growth on all surfaces tested. However, both *S. typhimurium* and *S. enteritidis* preferentially colonized polystyrene under same conditions as above (Figure 2).

Kinetics of biofilm formation on polystyrene

Biofilm growths on polystyrene followed different kinetics, being optimum at different times, 200 and 400 min for *S. enteritidis* and *S. typhimurium*, respectively (Figure 3).

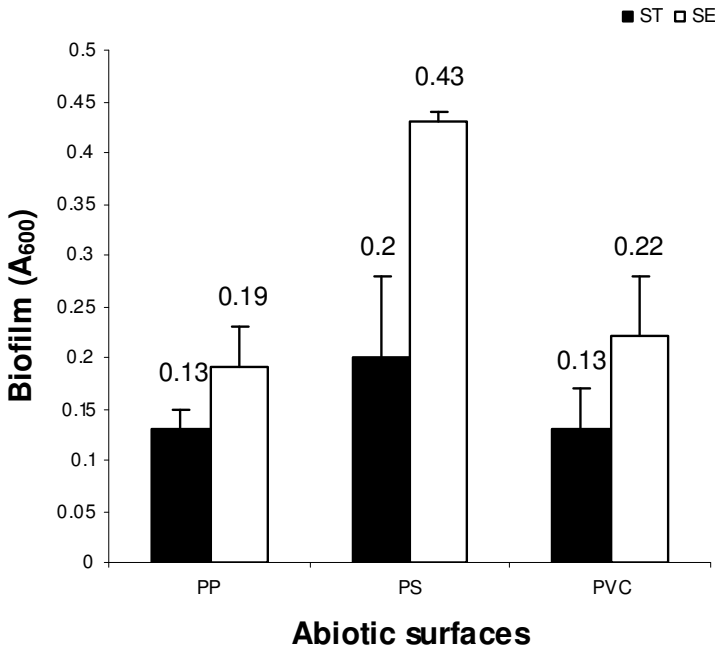

Figure 2. Biofilm formation on abiotic surfaces. Biofilm was assayed based on O`Toole and Kolter (1998). PP, Polypropylene= 1.5-ml microcentrifuge tube; PS, Polystyrene= 24-well tissue culture plate (FALCON); PVC, Polyvinyl chloride= 96-well microtiter plate; ST, *S. typhimurium* L1388; SE, *S. enteritidis* L1225. Error bars are standard deviations of the means of at least three determinations. Statistical significance was determined by ANOVA at P = 0.05.

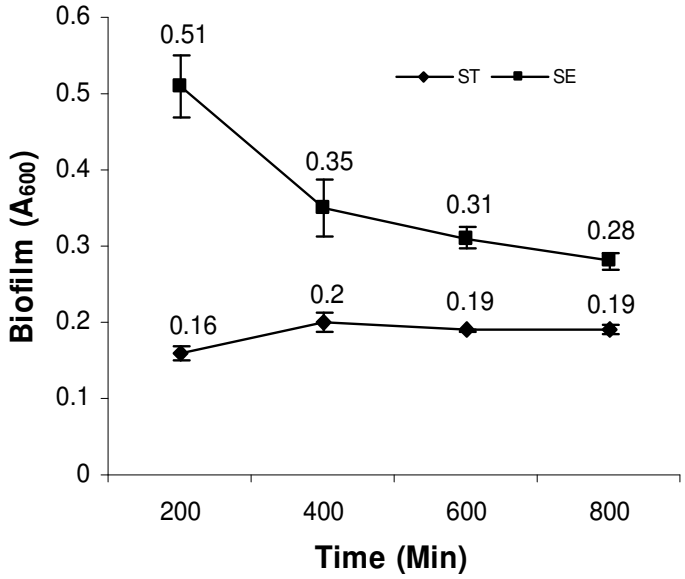

Figure 3. Time-course assay of biofilm growth on polystyrene by test strains. Biofilm growth on 24-well polystyrene Tissue culture plate was quantified by absorbance measurements (at 600 nm) of solublized CV-bound adhered cells following growth of bacteria in wells containing LB broth for the indicated times. ST, *S. typhimurium* L1388; SE, *S. enteritidis* L1225. Error bars are standard deviations of the means of at least three separate determinations. Statistical analysis was done using one-way ANOVA, and significance or otherwise, of differences was determined at P = 0.05.

Scanning electron microscopy

Both strain types formed biofilm in which bacterial cells aggregated together in microcolonies, attached to each other and to the polystyrene surface by an exopolymer substance (Figure 4). The thickness of the exopolymer was different, in favor of *S. enteritidis*.

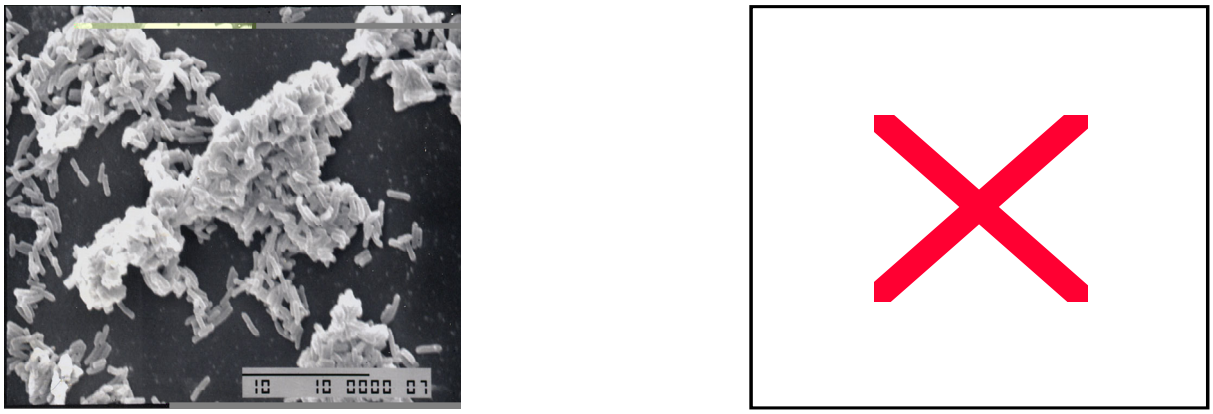

Figure 4. Scanning electron microscopy. Bacteria were grown (24 h, 37°C) in LB broth containing polystyrene chips (0.5 cm x 0.5 cm). The chips were fixed in buffered 3% glutaraldehyde, post-fixed in buffered 1% osmium tetroxide, dried in graded ethanol concentrations, vacuum-dried, sputter-coated with fine platinum and viewed with JSM-T300 SEM (JEOL Technics, Japan) at 10 kV. ST, *S. typhimurium* L1388; SE, *S.* enteritidis L1225. Scale bars = 10 µm.

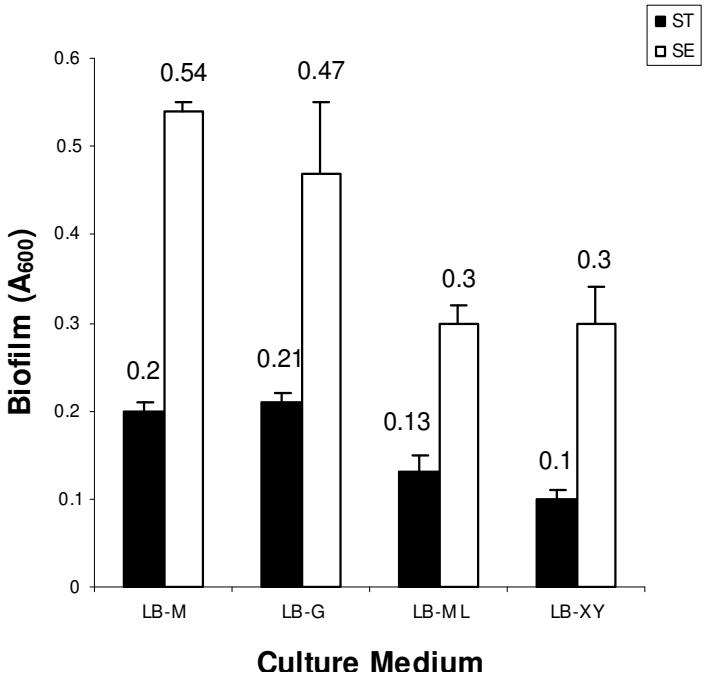

Figure 5. Effect of carbon sources on biofilm formation on polystyrene by bacteria. Biofilm was assayed based on O`Toole and Kolter (1998). LB-M, LB broth containing 100 mM D- (+)-Mannose; LB-G, LB broth containing 100 mM D- (+)-Glucose; LB-ML, LB broth containing 100 mM D- (-)-Mannitol; LB-XY, LB broth containing 100 mM D- (+)-xylose; ST, *S. typhimurium* L1388; SE, *S. enteritidis* L1225. Error bars are standard deviations of the means of at least three determinations. Statistical significance was determined by ANOVA at P = 0.05.

Effects of carbon source on biofilm formation

S. enteritidis formed significantly (P < 0.05) more biofilm than *S. typhimurium* when the growth medium was supplemented with 100 mM each of either D-(+)-mannose (P = 0.0001), D-(+)-glucose (P = 0.0005), D-(-)-mannitol (P = 0.00002) or xylose (P = 00009); however, biofilms formed by *S. enteritidis* grown in sugar-supplemented medium were insignificantly (P > 0.05) different from that grown in non-supplemented medium (Figure 5). Significant (P < 0.05) reduction in biofilm formed by *S. typhimurium* were produced by only mannitol (P = 0.0008) and xylose (P = 0.00004)

Effects of osmotic and starvation stresses on biofilm formation

Both *S. typhimurium* (P = 0.0084) and *S. enteritidis* (P = 0.0002) formed significantly (P < 0.05) less biofilm on polystyrene when grown for 24 h in LB-10%NaCl (osmotic stress) than in LB medium; and the quantity formed by *S. typhimurium* was significantly (P < 0.05: P = 0.0098) more than that by *S. enteritidis* (Figure 6). Similarly, both *S. typhimurium* and *S. enteritidis* formed significantly less biofilm on polystyrene when cultured in a starvation medium for 24 h.

Adherence to polystyrene

An insignificant difference was observed between *S. typhimurium* and *S. enteritidis* in their extent of adherence to polystyrene (Figure 7).

Chick mortality and oral median lethal dose (LD_{50})

Although *S. typhimurium* killed chicks more drastically than *S. enteritidis* in the first four days post inoculation,

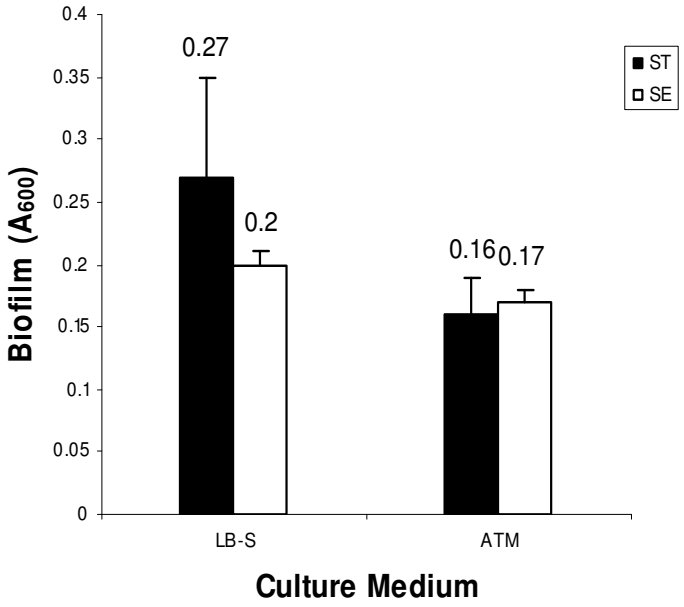

Figure 6. Effect of starvation and osmotic stress on biofilm formation on polystyrene by bacteria. Biofilm was assayed based on O`Toole and Kolter (1998). LB-S, LB with 10% NaCl; ATM, adherence test Medium (Gamazo et al., 1997). ST, *S. typhimurium* L1388; SE, *S. enteritidis* L1225. Error bars are standard deviations of the means of at least three determinations. Statistical significance was determined by ANOVA at P = 0.05.

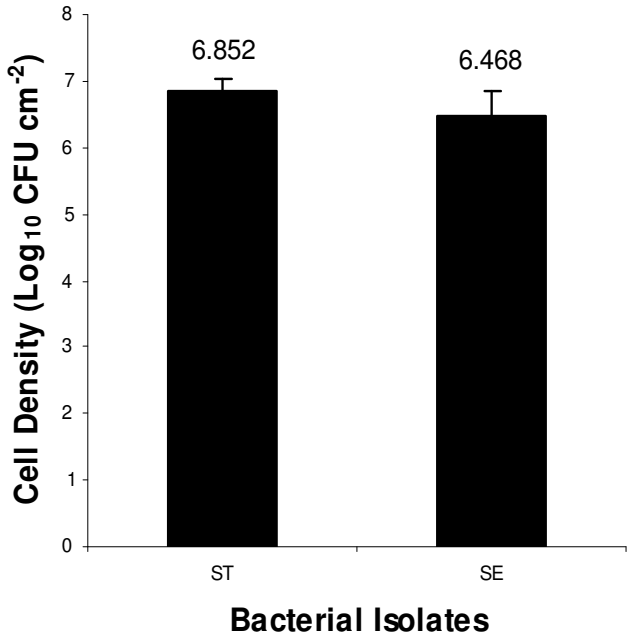

Figure 7. Adherence to polystyrene. Adherence describes the number of bacterial cells adhering to 1 cm^2 surface of polystyrene chips after overnight growth at 37°C (with shaking) in LB broth as described by Onaolapo et al. (1997). ST, *S. typhimurium* L1388; SE, *S. enteritidis* L1225. Error bars are standard deviations from mean of at least three determinations. Statistical significance was determined by ANOVA at P = 0.05.

the 14-day chick mortalities due to *S. typhimurium* and *S. enteritidis* were insignificantly ($P > 0.05$: $P = 0.3739$) different from each other. Oral LD_{50} for *S. enteritidis* was significantly higher than that of *S. typhimurium* (Table 2).

Table 2. Chick[a] mortality and oral median lethal dose (LD_{50}) of test strains.

Strain	Gram Body Weight (SD[b])	Dose (CFU)	4-day mortality				Mortality[1]	Median Lethal Dose[2] (Log_{10} LD_{50})
			Day 1	Day 2	Day 3	Day 4		
ST		5×10^6	0	0	6	3	9/10	< 2.699
	34.34	5×10^4	0	0	3	2	8/10	
	(2.82)	5×10^2	0	0	2	2	9/10	
SE		5×10^6	0	0	3	3	8/10	5.299
	31.40	5×10^4	0	0	2	0	5/10	
	(3.04)	5×10^2	0	0	1	3	7/10	

ST, *S. typhimurium* L1388; SE, *S. enteritidis* L1225. [1]Number of birds that died per dose level at day 14 post-infection/total numbers of birds infected. [2]Number (in logarithmic unit) of colony-forming units (CFU) of bacteria that killed 50% of Day-old White leghorn chicks randomized into groups of 10 and infected orally with test strains at the indicated three dose levels. Infected birds were observed two times daily for twenty-one days, during which the number of death chicks was recorded. The median lethal dose (LD_{50}) was estimated using the method of Reed and Muench (1938). A control group in which birds received only phosphate-buffered saline (pH 7.2) was included. Values in parentheses are standard deviations from mean. ST killed the birds more drastically than SE. [a]White Leghorn Chicks obtained from the National Institute of Animal Husbandry, Tsukuba, Japan. The chicks were handled in line with University's rules on animal experimentation.
[b]SD, standard deviation.

DISCUSSION

The ability of *Salmonella* to survive in wide varieties of environments is related to both their non-requirement for strict growth conditions and the capacity to form biofilm, a contributing factor to their success as pathogen (Kwon and Ricke, 1998; Solano et al., 1998). Since the infecting serotype influences the pattern of *Salmonella* disease, this study examined the *in vitro* colonization of the Typhimurium and Enteritidis serotypes under nutrient-rich, reduced water-activity and starvation culture conditions.

Although the serotypes had preference for similar colonizing surface, the greater capacity of the Enteritidis to form biofilm under nutrient-rich growth conditions on all the surfaces tested may be attributed to differences in bacterial cell surface appendages or physico-chemical factors operating at the colonizing surface (Fletcher, 1996). The difference in the surface colonization pattern of Typhimurium and Enteritidis, as indicated by their different kinetics of biofilm formation on polystyrene, suggest that Enteritidis is more likely to persist in its environment than Typhimurium.

Large deposit of exopolysaccharide (EPS) was observed in the Enteritidis biofilm on polystyrene as shown by scanning electron microscopy. This may account for the significantly larger amounts of biofilm formed by Enteritidis. EPS is important to microorganisms not only for initial attachment and firm anchorage of bacteria to solid surfaces (Oliveira et al., 1994), but also, for the maintenance of optimum environmental conditions by trapping and retaining nutrients for growth of biofilms (Kumar and Anand, 1998), protects bacteria from dehydration (Ophir and Gutnick, 1994) and critical for the persistence and survival in hostile environments, including effects of antimicrobial agents (Robertson and Firestone, 1992).

The lack of sensitivity of the biofilm formation on polystyrene by Enteritidis to changes of carbon sources including mannose suggests no possible role for Type 1 pili in Enteritidis' biofilm growth on polystyrene. Type 1 pili contain mannose-specific adhesin, FimH, which facilitates pathogenesis through specific interactions with mannose oligosaccharides present on eukaryotic cell surfaces (Old, 1972). Our observation contrasts with that of Austin et al. (1998) which advocated a role for Type 1 pili in the adherence to stainless steel or Teflon by *S. enteritidis*.

S. enterica grow optimally at water-activity (aw) = 0.99 (Taylor and McCoy, 1969). Osmotic drift due to the high salt concentration in the LB-10% NaCl medium resulted in a lower (than optimal) aw environment which is disruptive of normal cellular activities (Csonka and Epstein, 1996). Hence, the significantly less biofilm formed by the two serotypes on polystyrene when grown under reduced water-activity. This observation suggests that osmotic drift influences the responses of Typhimurium and Enteritidis biofilm growth on polystyrene in a similar manner.

The significant ($P < 0.05$) reduction in amounts of biofilm formed on polystyrene when both Typhimurium and Enteritidis were cultured in the starvation medium (Gamazo et al., 1997) shows that the formation of biofilm on polystyrene by both serotypes requires a nutrient-rich environment. This is in agreement with previous reports that biofilm formation is favored under most nutrient-sufficient environments (Fletcher, 1977; Wimpenny and Colasanti, 1997). Starvation is known to alter bacterial surface characteristics such as hydrophobicity, charge and irreversible attachment, which are essential factors in biofilm formation (Brown et al., 1977; Kjelleberg and Hermansson, 1984). The starvation medium used in this study is deficient in several essential nutrients, such as nitrogen, phosphorus, calcium, magnesium, sulfur, and iron, but with glucose as a source of energy. Since starv-

ation affected both serotypes in a similar manner, they could be speculated to possibly have a common starvation response pathway for biofilm formation on polystyrene. Our observation with polystyrene contrasts with the earlier report by Solano et al. (1998) that biofilm formation by *S. enteritidis* on glass surface was encouraged by the same starvation medium. This contrast could be explained by the fact that the effect of starvation on biofilm formation of *S. enterica* may be dependent on the surface being colonized.

Organisms involved in initial adhesion (an important determinant of the strength of biofilm adhesion) are only a small fraction of the number of organisms in a mature biofilm after growth (Busscher et al., 1995). Hence, the lack of correlation of bacterial adhesion with biofilm (shown by the insignificant difference in adhesion between the serotypes) may suggest the relevance of other factors to the biofilm process of these serotypes. It appeared that day-old chicks were more susceptible to Typhimurium than Enteritidis despite the greater capacity of the later to form biofilm on polystyrene *in vitro*.

Overall, the interaction of Typhimurium and Enteritidis with polystyrene does not correlate with virulence in young chickens. Greater understanding of how these serotypes interact with its host is needed to understand the disease and the colonization processes in chicken in terms of animal and public health.

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