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Attachment of *Streptococcus faecium* to the Duodenal Epithelium of the Chicken and Its Importance in Colonization of the Small Intestine

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The counts of *Streptococcus faecium* SY1 in the duodenums of gnotobiotic chicks exceeded the counts in their crops, indicating that multiplication was occurring in the anterior small intestine. This growth was related to adhesion to the gut wall which could be demonstrated by viable counts of macerated washed duodenal tissue. Scanning electron microscopy demonstrated that adhesion occurred in restricted areas on the surface of the villus, and transmission studies showed the presence of a thick extracellular layer on the bacterium. Attachment of *S. faecium* SY1 was confirmed in vitro by using chicken duodenal brush borders. The washings, produced during the preparation of the brush borders, increased the number of *S. faecium* adhering to the brush borders. This enhancing effect was due to the presence of trypsin in the duodenal washings. However, the effect was not dependent on the enzymatic activity of the trypsin molecule. The initial adhesion was not prevented by pretreatment of the brush borders with soy bean trypsin inhibitor. There were, therefore, two adhesion systems operating, only one of which was dependent on trypsin. Pretreatment of brush borders with trypsin digested them, but they remained intact in the presence of *S. faecium* SY1, indicating that the enzymatic activity was being inhibited. This effect was specific for the adhering strain of *S. faecium* SY1; the nonadhering *S. faecium* strain CRS23 and an adhering strain of *Lactobacillus* sp. were inactive, as was strain SY1 when adhesion was prevented by including sodium periodate in the test system. The colonizations of the gut by strains of *S. faecium* of differing adhesive abilities were compared. The nonadhering strain CRS23 showed reduced ability to colonize the duodenum, but the penicillin-resistant mutant of *S. faecium* SY1, which had reduced adhesive ability but could still attach to a lesser degree, was able to colonize the duodenum as efficiently as the parent strain.

In a previous study (S. B. Houghton, R. Fuller, and M. E. Coates, J. Appl. Bacteriol., in press), it was shown that the counts of *Streptococcus faecium* in the duodenums of chickens often exceeded those in their crops. This occurred in spite of the contents having to pass through the acid environment of the gizzard. It was proposed that the growth of *S. faecium* in the anterior small intestine was achieved by its attachment to the epithelial surface and, thus, its resistance to removal by peristalsis. This paper demonstrates the occurrence of adhesion and its importance in determining colonization of the small intestine.

MATERIALS AND METHODS

Chickens. All of the chicks tested were the progeny of Light Sussex hens crossed with Rhode Island Red cocks. Gnotobiotic chicks were hatched and reared in stainless steel isolators essentially by the methods described previously (5).

Bacteria. *S. faecium* strain SY1 is the strain previously described by Fuller et al. (13). *S. faecium* strain CRS23 was isolated on the tetrazolium-thallous acetate-glucose medium of Barnes (2) from a 7-day-old chick reared in a conventional brooder room. Strain KTM was a *Lactobacillus* sp., isolated on acetate agar (6), from the duodenal macerate of a 14-day-old bird reared in a conventional brooder room.

Viable counts. The contents of the crop, duodenum, ileum, and cecum were collected, diluted, and plated on yeast extract-glucose agar. The epithelium-associated flora was assessed by washing 1 cm of intestine, obtained from the apex of the duodenal loop, three times in phosphate-buffered saline (pH 7.2) and doing a viable count as described above on the third wash and on the tissue macerated in a Griffith tube. If the count in the macerate exceeded that in the third wash, adhesion was assumed to have taken place. Comparisons between means were made by *t* tests.

Electron microscopy. The methods previously described by Brooker and Fuller (3) were used.

Preparation of brush borders. Brush borders were prepared by the method of Sellwood et al. (23), except that the final filtration through glass wool was omitted.

Adhesion test. Preliminary tests were done to determine the best test conditions for measuring adhesion. It was found that a suitable ratio of *S. faecium* SY1 to duodenal brush borders was 100:1 at final concentrations of 10^7 and 10^5/ml, respectively. Cells from a 5-h culture of *S. faecium* SY1 showed better adhesion than those from a 24-h culture (9.6 and 6.3 bacteria per brush border, respectively, the average of five experiments counting 50 brush borders per experiment). Adhesion reached maximum by 30 min and remained stable up to 24 h.

The standard adhesion test consisted of mixing a 5-h culture of *S. faecium* SY1 and duodenal brush borders, from chicks 3 to 5 days old, in a ratio of 100:1 in ca. 0.25 ml of phosphate-buffered saline and incubating it at 37°C on a rotating platform (16 rpm) for 30 min. Adhesion was assessed by examining the cells by phase-contrast microscopy and counting the number of bacterial cells attached to the lumenal surfaces of 50 brush borders.

The effect of pretreatment on adhesion was expressed by calculating the number of bacteria per brush border as a percentage of the control value. This was referred to as the adhesion index (AI).

DW. Duodenal washings (DW) were produced during the preparation of the brush borders by passing ca. 2 ml of phosphate-buffered saline through the excised duodenal loop with a Pasteur pipette. The collected washings were centrifuged (300 × *g*) to remove the solid contents. The washings were added to the adhesion test system to give a final concentration of 40% (vol/vol).

Pretreatment of *S. faecium* and brush borders. The treatments used to pretreat *S. faecium* SY1 or duodenal cells were as follows (all enzymes and chemicals obtained from Sigma Chemical Co. unless otherwise stated): sodium periodate (British Drug Houses) at 10 mg/ml (pH 4.5), 5 min, room temperature; wheat germ lipase at 2 mg/ml (pH 7.5), 30 min, 37°C; protease at 0.2 mg/ml (pH 7.5), 15 min, 37°C; pepsin at 0.2 mg/ml (pH 2.8), 30 min, 37°C; papain at 2 mg/ml (pH 6.2), 30 min, room temperature; trypsin at 1 mg/ml (pH 7.2), 30 min, 37°C; mannan at 5 mg/ml (pH 7.2), 4 h, 37°C; araban at 5 mg/ml (pH 7.2), 4 h, 37°C (Koch-Light); inulin at 1 mg/ml, (pH 7.2), 30 min, 37°C; dextran at 10 mg/ml (pH 7.2), 30 min, 37°C; α-glucosidase at 3 mg/ml (pH 6.8), 30 min, 37°C; β-glucosidase at 2 mg/ml (pH 5.0), 30 min, 37°C; β-glucuronidase at 1 mg/ml (pH 6.5), 30 min, 37°C; α-amylase at 2 mg/ml (pH 6.9), 30 min, room temperature; hyaluronidase at 10 mg/ml (pH 7.2), 30 min, 37°C; dextranase at 0.3 mg/ml (pH 6.0), 30 min, 37°C; neuraminidase at 1 mg/ml (pH 5.0), 30 min, 37°C; *Lens culinaris* lectin at 2 mg/ml (pH 7.2), 20 min, room temperature; *Lotus tetragonolobus* lectin at 1 mg/ml (pH 7.2), 20 min, room temperature; *Pisum sativum* lectin at 1 mg/ml (pH 7.2), 20 min, room temperature; α-methyl-D-mannoside at 2 mg/ml (pH 7.2), 5 h, room temperature; α-L-fucose, *n*-acetyl-D-glucosamine, lactose, galactose, maltose, *n*-acetyl neuraminic acid, and α-methyl D-(+)-glucoside, each at 2 mg/ml (pH 7.2), 30 min, room temperature. Benzylpenicillin (Glaxo Ltd.) was included (1 μg/ml) in the yeast extract-glucose broth growth medium for the final 1 h of incubation at 37°C. After treatment the cells were washed once in phosphate-buffered saline. Additional treatments used to study trypsin-enhanced adhesion were: trypsin at 1.6 mg/ml (pH 7.2), 37°C; soybean trypsin inhibitor at 1.6 mg/ml (pH 7.2), 15 min, 37°C; chymotrypsin at 1.6 mg/ml (pH 7.8), 37°C; trypsinogen at 1.6 mg/ml (pH 7.2), 37°C. (British Drug Houses).

Trypsin agglutination test. From a standard dropping pipette (30 drops per ml) 1 drop of *S. faecium* SY1 culture was added to 8 drops of trypsin solution (2 mg/ml). The mixture was incubated overnight at room temperature and examined microscopically for clumping of the bacterial cells.

RESULTS

Colonization of the guts of gnotobiotic chickens by *S. faecium* SY1. The ability of *S. faecium* to colonize the small intestine was first demonstrated in conventional chicks (Houghton et al., in press). The study of the colonization of the guts of gnotobiotic chicks by *S. faecium* SY1 revealed that this strain colonizes the small intestine in a similar way (Table 1). The counts in the duodenums exceeded those in the crops on days 1, 2, 3, and 5 ($P < 0.001$, $P < 0.05$, $P < 0.05$, and $P < 0.1$, respectively). The count of *S. faecium* SY1 in the duodenal lumen and in the tissue macerate declined by 14 days. The high count of *S. faecium* in the tissue macerates suggests that a significant population is associated with the duodenum wall. This association was confirmed by scanning electron microscopy which showed the organism colonizing the surface of the intestine in discrete areas (Fig. 1). A network of interconnecting fibrils can be seen between the bacterial cells, and these probably also extend from the bacterial cell surfaces to the host plasma membrane (Fig. 2). The presence of an extracellular layer around *S. faecium* SY1 was shown by transmission electron microscopy (Fig. 3). The width of this layer is equivalent to the diameter of the cells, can be seen to be closely associated with the epithelial surface, and produced some distortion of the microvilli. The interconnecting fibrils seen by scanning electron microscopy were probably produced by shrinkage of the extracellular layer during preparation for scanning electron microscopy.

Characterization of adhesion determinants on *S. faecium* SY1. Initially, *S. faecium* SY1 was subjected to treatments specifically

TABLE 1. *Colonization of the intestine of gnotobiotic chicks by S. faecium strain SY1*

Source	Log_{10} colony-forming units[a] in chicks aged:					
	1 day	2 days	3 days	4 days	5 days	6 days
Contents[b]						
Crop	6.00 ± 0.59	6.72 ± 0.49	5.61 ± 0.35	6.03 ± 0.53	5.90 ± 0.42	5.65 ± 0.56
Duodenum	7.25 ± 0.05	7.43 ± 0.16	7.25 ± 0.15	6.73 ± 0.38	5.56 ± 0.33	5.00 ± 0.53
Ileum	8.71 ± 0.10	8.65 ± 0.18	8.49 ± 0.18	8.19 ± 0.56	6.88 ± 0.05	5.52 ± 0.17
Cecum	9.79 ± 0.06	9.73 ± 0.08	9.73 ± 0.23	9.74 ± 0.13	9.77 ± 0.05	6.85 ± 1.55
Duodenum tissue						
3rd wash[c]	4.11 ± 0.24	5.27 ± 0.49	5.06 ± 0.16	4.48 ± 0.41	3.62 ± 0.30	1.52 ± 0.31
Macerate[d]	3.92 ± 0.47	5.46 ± 0.33	5.55 ± 0.13	5.74 ± 0.60	4.77 ± 0.27	2.23 ± 0.21

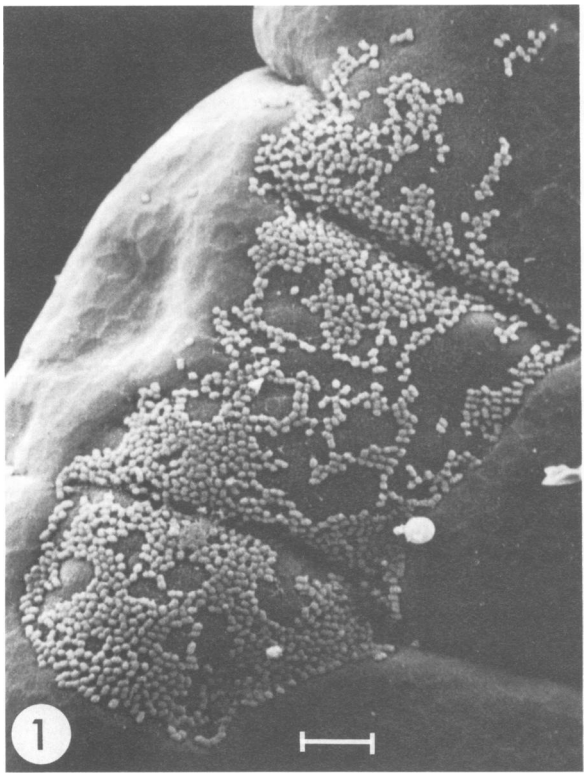
[a] Mean of three chicks ± standard error of the mean.
[b] Data given as log_{10} colony-forming units per gram (wet weight) of gut contents.
[c] Data given as the bacterial count (log_{10} colony-forming units) in the total wash volume.
[d] Data given as log_{10} colony-forming units in the macerate (from length of intestine of ca. 1 cm).

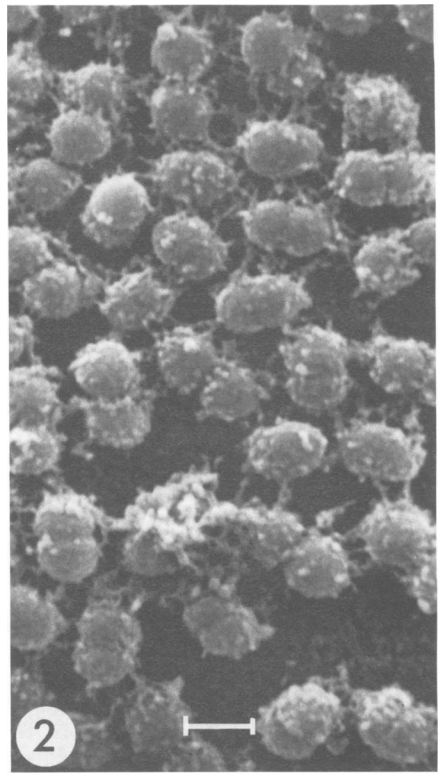
active against lipid, protein, or carbohydrate. Wheat germ lipase was without effect on adhesion, but protease showed a slight reduction. Treatment with sodium periodate for 5 min at pH 4.5, which is specific for carbohydrate (7), caused an almost total inhibition of adhesion (Table 2). It was concluded, therefore, that the adhesion determinant was a carbohydrate, and attempts were made to identify it further by using enzymes (hyaluronidase, dextranase, α-glucosidase, β-glucosidase, α-amylase, and neuraminidase). Of those tested, only α-glucosidase (AI = 84.3) and neuraminidase (AI = 78.1) gave more than 10% reduction of adhesion. Even so, the large reduction in adhesion obtained with periodate could not be repeated enzymatically. Pepsin and trypsin also gave no decrease in adhesion. Pretreatment with *L. tetragonolobus* lectin reduced the AI to 81.7, whereas lectin of *P. sativum* was completely inactive. The differences in the known binding specificities of these two lectins suggested various carbohydrates which might block the receptor on the epithelial cell. However, when these carbohydrates (α-L-fucose, D-(+)-galactose, *N*-acetyl galactosamine, α-lactose, maltose, neuraminic acid, and α-methyl glucoside) were tested by pretreating duodenal brush borders none reduced the AI below 95. Incorporation of 1 ppm of penicillin in the medium during the last hour of growth of *S. faecium* reduced the AI to 78.4.

Characterization of adhesion determinant on duodenal brush borders. The initial experiment showed that wheat germ lipase and periodate were without effect but that protease produced a slightly lowered AI of 88.4 (Table 2). A similar reduction was not produced by papain. Trypsin could not be tested because it digested the brush borders.

Adhesion enhancing effect of DW. Attempts to demonstrate the effect of gut secretions which might have been removed in the preparation of the brush borders were made by using the saline washings obtained during the preparation. When DW was added to the test system, there was a marked increase in adhesion of *S. faecium* SY1 to duodenal brush borders (Table 3). This effect could be reduced by dilution of the DW. To investigate whether the factor was a protein, the washings were treated with trypsin. The treatment with trypsin did not affect the adhesion stimulation by DW, but the trypsin control showed an increase in adhesion comparable to that obtained with DW. Also, the addition of soybean trypsin inhibitor to the system totally inhibited the effect of DW (Table 4). Chymotrypsin but not trypsinogen was as effective as DW in increasing adhesion. The respective AIs were 176.4 and 97.8. The addition of soybean trypsin inhibitor to the trypsin before introduction to the system removed the enhancing effect, but addition of soybean trypsin inhibitor or protease after the enhancing effect had occurred had no reversing effect (Table 4); trypsin stimulates adhesion at pH 4.0, which is outside its pH range of enzymatic activity. Treatment of trypsin with 8 M urea at pH 4.0 reduced the stimulatory effect (Table 4). Urea denatures trypsin and causes changes in conformation of its structure (17).

The adhesion of *S. faecium* SY1 to duodenal brush borders observed in the absence of added trypsin could not be attributed to membrane-bound trypsin on the surface of duodenal brush borders because pretreatment of the brush borders with soybean trypsin inhibitor did not reduce adhesion. The increased adhesion seen with trypsin might have been due to trypsin forming a bridge between attached cells and nonattached cells. However, this was discounted





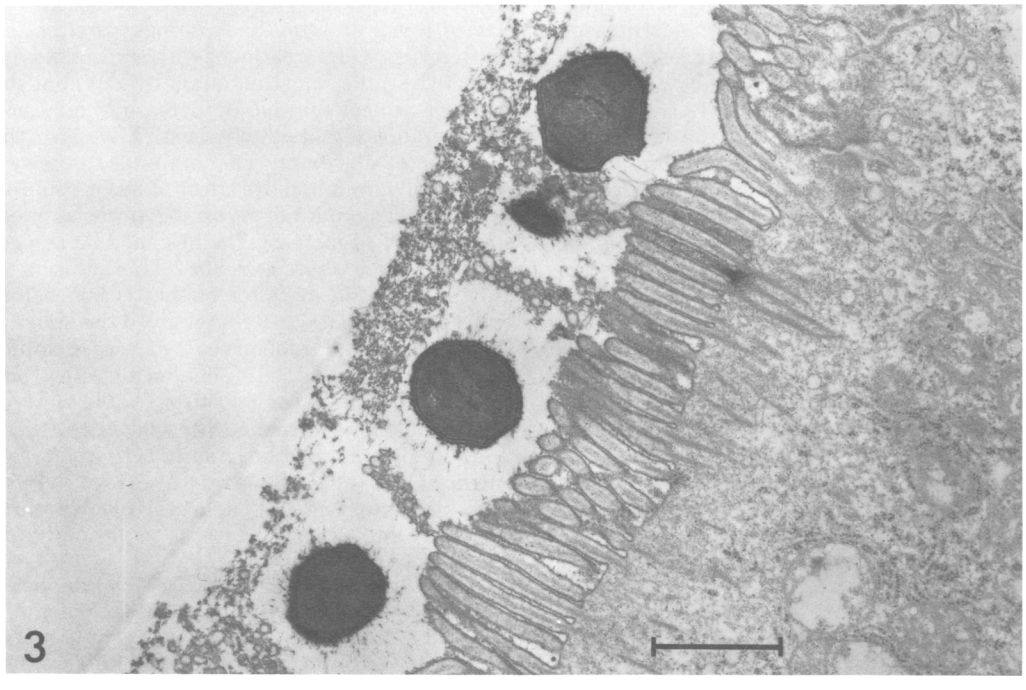


FIG. 1. *Scanning electron micrograph showing streptococci attached to the surface of a duodenal villus from a gnotobiotic chicken monoassociated with S. faecium SY1. Bar, 10 μm.*

FIG. 2. *Enlargement of part of Fig. 1 showing fibrils between cells. Bar, 1 μm.*

FIG. 3. *Transmission electron micrograph of streptococci attached to the duodenal brush border from a gnotobiotic chicken monoassociated with S. faecium SY1. The bacteria are surrounded by a lucent zone, indicating capsular material, in which there are fine fibrils which insert on the microvilli. Note the distortion of the microvilli adjacent to the attached bacterial cells. Glutaraldehyde-osmium tetroxide fixation in presence of ruthenium red. Bar, 1 μm.*

because trypsin did not agglutinate *S. faecium* SY1. There are, therefore, two receptors for *S. faecium* attachment on the duodenal epithelial cell.

Pretreatment of brush borders with trypsin digested them, but, in the presence of *S. faecium* SY1, the brush borders remained intact. This effect was specific for adhering strains of *S. faecium*; strain SY1 protected against digestion but strain CRS23 did not. Similarly, an adhering strain of lactobacillus also failed to protect against trypsin digestion. Moreover, if *S. faecium* SY1 was treated with sodium periodate (which inhibits adhesion), it lost its protective effect. These results suggested a trypsin-binding effect for strain SY1.

Relation between adhesion and colonization. *S. faecium* strain CRS23 isolated from the guts of chicks did not adhere to duodenal brush borders in vitro. The importance of adhesion in colonization was demonstrated by comparing the establishment of *S. faecium* SY1 and *S. faecium* CRS23 in gnotobiotic chicks (Table 5). *S. faecium* SY1 showed a significantly higher duodenal count in both the lumen and the tissue at day 3 ($P < 0.001$).

TABLE 2. *Effect on adhesion of pretreating S. faecium and duodenal brush borders*

Treatment	No. of bacteria/brush border	
	S. faecium SY1	Brush borders
Wheat germ lipase	4.1 (121)[a]	11.3 (122)
Protease	4.8 (89)	8.5 (88)
Sodium periodate	0.7 (11)[b]	8.22 (99)

[a] Numbers within parentheses are the number of bacteria per brush border expressed as a percentage of the value for untreated bacteria or brush borders.
[b] Mean value of four experiments, each experiment being the mean count of bacteria adhering to 50 brush borders.

TABLE 3. *Adhesion-enhancing effect of DW*

DW concn (vol/vol)	No. of expt	Adhesion of *S. faecium* SY1 (mean no. of bacteria/brush border)	
		Treated	Untreated
80	3	14.0 (237)[a]	5.9
40	2	15.8 (221)	7.2
30	3	10.6 (149)	7.5
20	1	11.2 (189)	5.9
10	1	7.1 (119)	5.9

[a] Numbers within parentheses are the number of bacteria per brush border expressed as percentage of the value for the untreated test system.

TABLE 4. *Characterization of adhesion-enhancing ability of duodenal washings and trypsin*

Treatment	Adhesion of *S. faecium* SY1 (mean no. of bacteria/brush border)[a]	
	Treated	Untreated
DW	15.8 (221)[b]	7.2
Trypsin	15.4 (215)	7.2
DW + SBTI[c]	7.9 (111)	7.2
Trypsin + SBTI	6 1 (85)	7.2
SBTI added to brush borders before *S. faecium* SY1	5.9 (88)	6.7
SBTI added after adhesion	13.8 (212)	6.5
Protease added after adhesion	13.0 (245)	5.3
Trypsin at pH 4.0	13.3 (175)	7.6
Trypsin treated with urea at pH 4.0 before adhesion	8.3 (109)	7.6

[a] Mean of two experiments.
[b] Numbers within parentheses are the number of bacteria per brush border expressed as a percentage of the value of the untreated test system.
[c] SBTI, Soybean trypsin inhibitor.

TABLE 5. *Colonization of the intestine of gnotobiotic chicks by adhering (SY1) and nonadhering (CRS23) strains of S. faecium*

Source	Log_{10} colony-forming units[a] of SY1 in chicks aged:		Log_{10} colony-forming units[a] of CRS23 in chicks aged:	
	3 days	14 days	3 days	14 days
Contents[b]				
Crop	6.65 ± 0.45	6.47 ± 0.14	4.05 ± 0.64	7.19 ± 1.16
Duodenum	7.18 ± 0.47	4.35 ± 0.78	3.69 ± 0.28	5.08 ± 0.71
Duodenum tissue				
3rd wash[c]	5.08 ± 0.47	<2.00 ±ND[d]	2.70 ± 0.35	2.23 ± 0.23
Macerate[e]	6.39 ± 0.50	2.30 ± 0.73	2.58 ± 0.61	3.04 ± 0.43

[a] Mean of three chicks ± standard error of the mean.
[b] Data are given as log_{10} colony-forming units per gram (wet weight) of gut contents.
[c] Data are given as the bacterial count (log_{10} colony-forming units) in total wash volume.
[d] ND, not determined; counts were below limit of technique used.
[e] Data are given as log_{10} colony-forming units in macerate (from length of intestine of ca. 1 cm).

The possibility was considered that there is some change either in duodenal cells or in *S. faecium* SY1 which might result in the decreased adhesion seen in gnotobiotic chicks over a 14-day period. Duodenal brush borders were prepared from 5-day- and 14-day-old germfree chicks, and *S. faecium* SY1 was isolated from 3- and 14-day-old monoassociated chicks. When tested against brush borders from 5-day-old chicks, *S. faecium* SY1 showed good adhesion whether isolated from 3-day- or 14-day-old chicks (the AIs were 111 and 103, respectively, compared with 100 for control *S. faecium* SY1 used in the standard test). *S. faecium* SY1 did not show reduced adhesion to duodenal brush borders from 14-day-old chicks, the AI being 117 with 14-day-old brush borders compared with 100 for 5-day-old brush borders (which is equivalent to that of the standard adhesion test control). It seems, therefore, that the reduction in numbers of *S. faecium* SY1 associated with the duodenum is not a reflection of change in the adhesive ability of SY1 or duodenal cells. It may be that it reflects the attempts of the chick to control the *S. faecium* population by secretory antibody or some other antibacterial agent.

The observation that the inclusion of penicillin in the growth medium had an effect on adhesion of *S. faecium* SY1 to duodenal brush borders was interesting because it could explain the effect of penicillin on numbers of *S. faecium* in the gut and the growth response of chicks. A mutant of *S. faecium* SY1 resistant to penicillin (10 ppm) was selected, and its adhesion to duodenal brush borders was compared with the normal sensitive *S. faecium* SY1 in vitro. In the absence of penicillin, the resistant strain showed a marked decrease in adhesion compared with the sensitive parent strain (AI = 59.2, mean of three experiments). The inclusion of penicillin (1 ppm) in the test system had no effect on adhesion; the respective values for the sensitive strain in the presence and absence of penicillin were 11.1 and 11.3 bacteria per brush border compared with 6.0 and 5.3 for the resistant strain. A comparison was made between the ability of resistant and sensitive *S. faecium* SY1 to colonize the small intestine, but no difference could be demonstrated in the ability to colonize the gut (Table 6). Tests on isolates from the gnotobiotic chicks made at the end of the experiment showed that the resistant strain was still resistant to 10 ppm of penicillin.

In a previous study (Houghton et al., in press), it was shown that anaerobically grown *S. faecium* SY1 was more growth depressing for gnotobiotic chicks than was aerobically grown SY1. When tested for adhesion in vitro, the AIs for aerobically and anaerobically grown *S. faecium* SY1 were 4.2 and 6.6, respectively (mean of three experiments). Therefore, we considered the possibility that the increased growth-depressing ability of the anaerobic *S. faecium* SY1 was due to improved adhesion to the gut wall by the anaerobic culture resulting in the production of increased numbers of *S. faecium* in the small intestine (Table 7). However, at day 3 there was no difference between the group of chicks given an anaerobic culture and those given the aerobic culture. Indeed, at day 14 the counts of *S. faecium* in the chicks given the aerobic culture were higher in their crops ($P < 0.02$), duodenums ($P < 0.05$), and duodenal macerates ($P < 0.1$). The difference in growth-depressing capacity of aerobically and anaerobically grown *S. faecium* was not explainable in terms of improved colonization of the gut lumen or gut wall.

DISCUSSION

It has been shown that *S. faecium* SY1 colonizes the duodenum wall and that it adheres to

TABLE 6. *Colonization of the intestine of gnotobiotic chicks by penicillin-sensitive (PS) and -resistant (PR) strains of S. faecium SY1*

Source	Log_{10} colony-forming units[a] of PS strains in chicks aged:		Log_{10} colony-forming units[a] of PR strains in chicks aged:	
	3 days	14 days	3 days	14 days
Contents[b]				
Crop	6.75 ± 0.62	5.24 ± 0.09	6.46 ± 0.20	6.50 ± 0.33
Duodenum	7.29 ± 0.45	5.35 ± 0.87	7.03 ± 0.37	4.22 ± 0.44
Duodenum tissue				
3rd wash[c]	3.72 ± 0.71	<2.0 ± ND[d]	3.36 ± 0.71	<2.0 ± ND
Macerate[e]	5.53 ± 0.57	3.10 ± 0.10	4.84 ± 0.94	3.0 ± ND

[a] Mean of three chicks ± stndard error of the mean.
[b] Data are given as log_{10} colony-forming units per gram (wet weight) of gut contents.
[c] Data are given as the bacterial count (log_{10} colony-forming units) in the total wash volume.
[d] ND, not determined; counts were below limit of technique used.
[e] Data are given as log_{10} colony-forming units in macerate (from a length of intestine of ca. 1 cm).

TABLE 7. *Colonization of the intestine of gnotobiotic chicks by S. faecium SY1 grown aerobically and anaerobically*

Source	Log_{10} colony-forming units[a] of aerobic SY1 in chicks aed:		Log_{10} colony-forming units[a] of anaerobic SY1 in chicks aged:	
	3 days	14 days	3 days	14 days
Contents[b]				
Crop	6.74 ± 0.69	6.40 ± 0.53	6.80 ± 0.67	5.19 ± 0.44
Duodenum	7.19 ± 0.17	5.40 ± 0.97	7.15 ± 0.17	4.05 ± 0.45
Duodenum tissue				
3rd wash[c]	5.03 ± 0.25	2.46 ± 0.38	5.06 ± 0.31	2.33 ± 0.34
Macerate[d]	5.57 ± 0.22	3.52 ± 0.87	5.79 ± 0.32	2.63 ± 0.18

[a] Mean of three chicks ± standard error of the mean.
[b] Data are given as log_{10} colony-forming units per gram (wet weight) of gut contents.
[c] Data are given as the bacterial count (log_{10} colony-forming units) in the total wash volume.
[d] Data are given as log_{10} colony-forming units in the macerate (from a length of intestine ca. 1 cm).

duodenal brush borders in vitro and in vivo. The importance of adhesion in the colonization of the gut was shown by comparing the establishment of adhering (SY1) and nonadhering (CRS23) strains of *S. faecium* in the guts of gnotobiotic chicks. *S. faecium* CRS23 did not adhere to duodenal brush borders in vitro and was not as good a colonizer of the small intestine as *S. faecium* SY1, which does adhere to duodenal brush borders. The importance of adhesion in colonization of the mouth and gastrointestinal tract has been shown in a number of previous studies (16, 22). In chickens, it has been shown that lactobacilli that adhere to crop epithelial cells are better able to colonize the crop. The high numbers in the crop are reflected in the small intestine (12). Nonindigenous (presumably nonadhering) strains of lactobacilli colonize the guts of gnotobiotic chicks as monoassociates but are suppressed by indigenous (presumably adhering) chicken isolates (19). Similarly, in pigs, the K88 antigen is necessary for the attachment of *Escherichia coli* to the small intestine; without attachment, *E. coli* cannot produce diarrhea (18).

The decline in numbers of *S. faecium* SY1 in the duodenum of a gnotobiotic chicken during the first 14 days of life could not be attributed to changes in the adhesive capabilities of either the chick cells or *S. faecium* SY1. Immune competence is developing during this period, and the changes may be due to antibody in the same way that secretory immunoglobulin A is involved in preventing the adhesion of streptococci to human buccal cells (26) and the adhesion of *Vibrio cholerae* to the intestinal mucosa (10, 11). Secretory antibody has also been suggested as an explanation for the fall in the number of *E. coli* in rabbit guts (4) and has been shown to prevent adhesion of *E. coli* to human urinary tract epithelial cells (25).

Electron microscopy showed that *S. faecium* SY1 colonizes the surface of the crop epithelium as discrete microcolonies. *E. coli* colonizes the small intestine of pigs as microcolonies (18) as does *Streptococcus faecalis* on rat tongue papillae (15). The reason for this pattern of colonization is not clear. The colonization of specific cell types is an unlikely explanation, since 95 to 100% of brush borders in in vitro preparations have receptors for *S. faecium*. Attachment and growth may only occur where a protective layer is breached. The lack of a confluent cover of bacteria may reflect a host response to the presence of the bacteria, host suppression of the bacterial population by local antibody production, or the inability of the microflora to grow fast enough to colonize the entire surface before desquamation occurs.

The reduction of adhesion, obtained by growing *S. faecium* SY1 for short periods in the presence of penicillin, may help to explain the growth-promoting effect of dietary penicillin for chicks. Subinhibitory concentrations of antibiotics reduce the adhesion of *E. coli* to human buccal epithelial cells in vitro (8) by interfering with protein synthesis and thereby reducing piliation. Streptomycin-resistant strains of *Streptococcus mutans* colonize rat guts less well than sensitive strains (1). A penicillin-resistant mutant of *S. faecium* SY1 did not adhere as well to duodenal brush borders in vitro as the sensitive strain. This difference could not be reproduced in vivo. This contrasts with the findings with strains SY1 and CRS23 which gave a good correlation between in vitro and in vivo adhesive ability. Penicillin resistance does not completely suppress adhesion, and the residual adhesive ability may be sufficient to ensure colonization of the intestine. Similarly, although aerobically grown strain SY1 attaches less well in vitro to brush borders than anaerobically grown SY1, it

attaches equally well in vivo and colonizes the small intestine as effectively as the anaerobic culture.

The enhancement of adhesion by DW highlights one of the dangers that must be recognized in systems with brush borders. The preparation of the test epithelial cells may wash off factors which either are responsible for or protect against attachment. In the present case, the presence of trypsin in the washings was shown to be responsible for the stimulation of adhesion. This effect was not due to the enzymatic action of the trypsin because trypsin was just as effective in stimulating adhesion at pH 4.0 as at pH 7.2. The adhesion observed in the absence of added trypsin could not be attributed to trypsin residues bound to the duodenal brush border surface because treatment of the brush borders with soybean trypsin inhibitor before addition of SY1 cells did not reduce adhesion. There are, therefore, two adhesion systems present in the chick gut, one trypsin dependent and one trypsin independent.

Preparation of the brush borders also made them susceptible to trypsin digestion, which does not occur in vivo. *S. faecium* SY1 protects the brush borders against tryptic digestion and is presumably binding trypsin. However, this could not be confirmed by measuring tryptic activity. The protection of brush borders against tryptic digestion was a specific effect; *S. faecium* strain CRS23, which did not adhere to brush borders, was inactive as was a *Lactobacillus* strain which did adhere. Moreover, rendering the *S. faecium* SY1 nonadhesive with sodium periodate destroyed its protective effect.

The common feature of adhesion determinants on bacterial surfaces is that they are extracellular in the form of fimbriae as in *E. coli* (9, 24) or capsular material as in the case of *Bacteroides fragilis* (21) or *Lactobacillus salivarius* (3). *S. faecium* SY1 has been shown to produce extracellular material in vivo, and it is probably a carbohydrate within this layer that is involved in adhesion.

The lack of success in identifying the adhesion determinant on the surface of the duodenal brush borders is disappointing. Some studies have identified host cell adhesion determinants. For example, *E. coli* binds to mannose moieties on the surface of human epithelial cells (20) and β-D-galactosyl residues on intestinal cells have been suggested as receptors for the K88-mediated adhesion of *E. coli* (14).

The attachment of *S. faecium* to duodenal brush borders has been demonstrated, and it is suggested that this may be a factor in determining whether a strain is effective in producing growth depression of chickens. Attachment is increased by trypsin, and the protection by *S. faecium* by brush borders from tryptic digestion suggests a trypsin-binding function. The interaction of attached *S. faecium* and protein digestion will be studied in relation to the effect of *S. faecium* on growth of chickens.

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