Studies on the Intestinal Microflora of Chicken Under Tropical Condition

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ABSTRACT : Three media, i. e., MOD-SD, M98-5 and M98-5 supplemented with chicken fecal extract were tested as isolation media for anaerobic bacteria present in the duodenum, jeju-ileum and cecum of chicken. The results showed that the mean colony counts of medium M98-5 were similar with those of MOD-SD medium in all intestinal samples at the incubation periods of 2, 6 and 10 days. Supplementation with chicken fecal extract of M98-5 medium significantly increased ($p < 0.05$) the colony counts of bacteria from the duodenum, jeju-ileum and cecum. The colony counts at 6-day incubation were similar with those at 10-day incubation, but were much higher than the counts at 2-day incubation.

The major types of bacteria found in the duodenum and jeju-ileum of chicken were tentatively identified as *Lactobacillus, Streptococcus* and *E. coli*. In the cecum, ten tentatively identified groups of bacteria, namely, *Streptococcus, Staphylococcus, Lactobacillus, E. coli*, anaerobic coccus, *Eubacterium, Propionibacterium, Clostridium, Fusobacterium* and *Bacteroides* were isolated. Anaerobes were found to comprise nearly the entire microbial population of the cecum. Predominating in all sections of the intestine were homofermentative lactobacilli. The main *Lactotacillus* species in chicken intestine were *L. acidophilus, L. fermentum* and *L. brevis*.

(**Key Words**: Chicken, Intestine, Bacteria, Lactobacillus, Media)

INTRODUCTION

The study on alimentary microbial flora in chicken dates back to the beginning of this century, but knowledge in this field is still lacking, particularly on the alimentary microflora of poultry under warm and humid tropical conditions.

One of the constraints in the study of microflora of poultry is the selection of appropriate media for growth and maintenance of the microflora. A few types of recovery media for poultry intestinal bacteria, such as rumen-fluid-glucose-cellobiose agar (RGCA) (Bryant and Burkey, 1953), M98-5 (Bryant and Robinson, 1961) and Medium 10 (Caldwell and Bryant, 1966) have been developed. Media developed for isolation of rumen bacteria and sludge digestor anaerobes have been used for the recovery of chicken cecal bacteria from 5-week-old birds (Salanitro et al. 1974a), and the highest colony counts occurred on medium M98-5. Later, Kelley (1983) found that M98-5 was less effective than RGCA-based media for isolation of cecal bacteria in 2- or 3-week-old turkey poults, but was equal or better than the RGCA-based media in 6-week-old birds. Recently, a rumen fluid-based differential carbohydrate agar medium for enumerating chicken cecal carbohydrate-utilizing bacteria was reported by Fan et al. (1995). Another medium (Scott and Dehority, 1965) which has been used successfully for isolating anaerobic rumen bacteria can be adapted for isolation of intestinal bacteria of poultry.

The objective of this study was to compare the suitability of Scott and Dehority's medium and M98-5 medium with or without chicken fecal extract for isolation of intestinal anaerobic bacteria, and to isolate and identify the bacteria from the duodenum, jeju-ileum and cecum of chicken under tropical conditions.

MATERIALS AND METHODS

Animals and diet

Three to five-week-old Arbor Acres broilers obtained from the university's experimental farm were reared in wooden cages under natural lights. The ambient temperature ranged from 24℃ - 34℃ and the relative humidity was approximately 70-100%. The chickens had free access to feed and water. The composition of the basal diet was the same as that described by Jin et al. (1996).

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Fecal extract preparation

Equal quantities of chicken feces and distilled water were mixed and autoclaved at 121℃, 15psi for 20 min. The sludge was centrifuged twice at 10,000 xg for 10 min and the supernatant obtained was adjusted to pH 7.0-7.2, autoclaved (121℃, 20min), and stored in the freezer at −20℃ until used for preparation of medium FM98-5.

Rumen fluid preparation

Rumen fluid obtained from a fistulated buffalo was filtered through double layers of cheese cloth. The filtered rumen fluid was autoclaved at 121℃ for 20 min, and centrifuged twice at 10,000 xg for 20 min. The clarified rumen fluid was stored at −20℃ until used for preparation of all the 3 media.

Anaerobic culture technique

Strict anaerobic techniques were used in all procedures involving dilution and inoculation of samples and preparation of media. Anaerobic techniques employed were similar to those described by Hungate (1969) for rumen bacteria with modifications by Bryant and Burkey (1953).

Preparation of media

Three media, i. e., Scott and Dehority's artificial modified medium (MOD-SD) (Scott and Dehority, 1965), M98-5 (Bryant and Robinson, 1961), and M98-5 + Fecal extract (FM98-5), were used to compare their suitability for isolating anaerobic bacteria in chicken intestine. The compositions of these media are given in table 1. M98-5 medium was modified from 98-5 medium (Bryant and Robinson, 1961) for culturing bacteria from anaerobic sludge digesters. It had an addition of glycerol, trypticase and hemin, and mineral solutions I and II were substituted with mineral solution S2. Scott and Dehority's artificial modified medium (MOD-SD) contained a mixture of volatile fatty acids, hemin, casein, vitamin mix as well as rumen fluid.

In the preparation of the three media, dilution blank solution (0.225 g K_2HPO_4, 0.225 g KH_2PO_4, 0.225 g $(NH_4)_2SO_4$, 0.45 g NaCl, 0.092 g $MgSO_4 \cdot 7H_2O$ and 0.06 g $CaCl_2 \cdot 2H_2O$/L) and peptone-yeast extract-glucose (PYG) medium (5 g peptone, 5 g trypticase, 10 g yeast extract, 10 g glucose, 10 ml hemin solution, 0.2 ml vitamin K1, 0.5 ml L-cysteine. HCl and 4 ml salt solution), all the ingredients used, except Na_2CO_3 and cysteine, were dissolved in distilled water, and the solutions adjusted to pH 6.8 - 7.0 using 1 N NaOH or HCl. Fifty ml of 8% (w/v) Na_2CO_3 were added and the volume was made up to 1 L. L-cysteine (3 g) was added after the medium was boiled, then CO_2 was bubbled through the medium for 5 min and allowed to cool for another 5 min under CO_2. The medium (9 ml) was then dispensed into test tubes and stoppered tightly. All the media were autoclaved at 121℃ for 20 min. For agar media, 20 g agar were included in 1 L medium.

Table 1. Composition of media (per liter)*

Component	MOD-SD	M98-5	FM98-5
Rumen fluid (ml)	400	400	400
Mineral S2 (ml)†	–	50	50
Mineral I (ml)†	200	–	–
Mineral II (ml)†	200	–	–
Hemin (ml)§	1	1	1
Casein (ml)	20	–	–
Rasazurin (ml)¶	1	1	1
Vitamins (ml)#	10	–	–
VFA (ml)#	66.7	–	–
Tripticase (g)	–	2.0	2.0
Soluble starch (g)	–	0.3	0.3
Glucose (g)	4.5	0.3	0.3
Cellobiose (g)		0.3	0.3
Maltose (g)		0.3	0.3
Glycerol (g)		0.3	0.3
Fecal extract (ml)			100
$Na_2S \cdot 9H_2O$ (g)	–	0.25	0.25
Agar (g)	20	20	20

MOD-SD, Scott and Dehority medium; FM 98-5, M98-5 + fecal extract;

* Final pH 6.8-7.0; 100% CO_2 gas phase;

† Mineral S2 [0.82 g KH_2PO_4, 18.12 g NaCl, 1.82 g $MgSO_4 \cdot 7H_2O$, 0.59 g $CaCl_2 \cdot 2H_2O$, 2.91 g $(NH_4)_2SO_4$, 3.24 g $MnCl_2 \cdot 4H_2O$ and 0.38 $CoCl_2 \cdot 6H_2O$/L];

† Mineral I [4.5 g KH_2PO_4/L], Mineral II [4.5 g NaCl, 4.5 g $(NH_4)_2SO_4$, 0.25 g $CaCl_2 \cdot 2H_2O$, 0.25 g $MgSO_4 \cdot H_2O$, 0.1 g $FeSO_4 \cdot 7H_2O$, 0.1 $ZnSO_4 \cdot 7H_2O$ and 0.01 g $CoCl_2 \cdot 6H_2O$/L];

§ Hemin, 0.0001 M in 0.005 M NaOH;

¶ Resazurin, 0.1% in distilled water;

VFA [20 ml acetic acid, 1.0 ml isobutyric, 1.2 ml isovaleric, 1.2 ml N-valeric and 1.2 ml 2-methyl-butyric/L], Vitamins [0.2 g pyridoxine · HCl, 0.2 g riboflavin, 0.2 g thiamine · HCl, 0.2 g nicotinamide, 0.2 g Ca-D-pantothenate, 0.01 g para-amino-benzoic acid and 1.0 ml stock solution (0.125 g folic acid, 0.125 g biotin and 0.0125 cobalamine/25 ml)/L].

Enumeration of intestinal bacteria

Direct microscopic clump counts for intestinal bacteria were made from intestinal contents according to the procedure described by Holdeman and Moore (1975). Bacterial colony counts in roll tubes with the three different media were also made after 2, 6 and 10 days of incubation. Colonies were counted using a colony counter

(Funke, Germany). Direct microscopic clump counts and colony counts were corrected for actual sample size based on dry weight of sample. Dry matter of intestinal samples was determined by drying approximately 1.0 g of sample in an oven at 90℃ until constant weight. All the experiments were conducted three times, each time two chickens were sacrificed.

Preparation of intestinal samples

Broilers were heavily anaesthetized and sacrificed by bleeding the jugular vein. Approximately 1 g of the mixed duodenal, jeju-ileal and cecal contents were taken immediately and placed in separate sterile test tubes containing 9 ml of sterile dilution blank solution. The remainder of the intestinal contents was used for dry matter determination. The samples were serially diluted under anaerobic conditions to 10^{-6} for duodenal content, 10^{-7} for jeju-ileal content and 10^{-9} for cecal content. For isolation of anaerobic bacteria, 0.1 ml of each dilution was added to separate tubes containing pre-reduced agar medium in a molten form maintained at 50℃. The tubes were rolled in a spinner till the medium solidified around the tube. They were then incubated at 39℃ for up to 10 days. For aerobic bacteria, 0.1 ml of each dilution was plated onto brain heart infusion agar medium (BHIA, Oxoid). The plates were incubated at 39℃ for 2 days after which the total number of colonies on each plate was recorded.

Identification of intestinal bacterial strains

Bacterial colonies were isolated from the agar roll tubes described above and the bacterial isolates were subcultured into tubes containing 3 ml of pre-reduced PYG medium. The isolates were incubated for 7 days at 39℃ after which their pH values were determined with an electronic pH meter and their fermentation end-products such as volatile fatty acids and catalase analyzed.

Analyses of fermentation products

Volatile fatty acids (VFA) which included acetic, propionic, butyric, iso-butyric, valeric and iso-valeric acids; non-VFA consisting of lactic and succinic acids formed in PYG medium were analyzed by gas chromatography (Shimadzu Model GC-14A) fitted with a flame ionization detector (FID) and nitrogen as the carrier gas with a flow rate of 60 ml/min. The column for VFA was 10% PEG 6,000 on Shimalite TPA 60/80 and the running conditions were 160℃ with FID at 230℃ and injector at 230℃. The internal standard used was 20 mM 4-methtyl-n-valeric acid. The non-VFA (lactic and succinic acids) were analyzed using a 1% PEG 20M on Tenax GC 80/100 column and the running conditions were 180℃ with FID at 190℃ and injector at 190℃. The internal standard was 20 mM fumaric acid.

Isolation and identification of *Lactobacillus* from the intestine

Five 3-week-old broilers were heavily anaesthetized and sacrificed by bleeding the jugular vein. After the body cavity was opened, the intestine was ligated at the duodenal, jeju-ileal and cecal regions. Tissue samples from the jeju-ileum and cecum were washed by inversion 10 times, and then homogenized in phosphate buffered saline (PBS) using a blender. The mixture was diluted and cultured onto Man-Rogosa-Sharpe (MRS) Agar (BBL, USA) plates. The plates were placed in a GasPak system (BBL, USA) and incubated anaerobically for 48 h at 37℃. Colonies picked from countable plates were inoculated into tubes containing 10 ml of sterile MRS broth (BBL, USA), and also onto MRS agar plates which were incubated anaerobically in a GasPak system (BBL, USA).

Gram-positive and catalase-negative rods were selected for further identification. Tests for catalase were made by adding 5 ml of 3% hydrogen peroxide to the cell pellet obtained by centrifuging (3,000 xg for 10 min at 4℃) 10 ml of a MRS broth culture. Cultures were considered catalase negative if no visible gas production was observed.

Cultures of the Gram positive bacteria and the catalase negative rods were examined for growth at 15℃ and 45℃ in MRS broth and for gas production using the methods of Rogosa and Sharpe (1959); for the latter, 5 ml of 1.5% sterile agar was used as the overlay. The ability of cultures to ferment various carbohydrates was evaluated by the API kit system (bioMérieux, France). The isolates were grown in MRS medium (BBL, USA) overnight and centrifuged for 10 min at 3,000 xg. The pellets were washed with PBS twice and transferred into API CHL 50 (bioMérieux, France) medium. The resuspended bacteria were inoculated into each unit of the API 50 kit (bioMérieux, France), and incubated anaerobically at 37℃. The color change in each unit was recorded every 24h for 7 days. Species of *Lactobacillus* were identified using a computer program (APILAB, France). All the identified strains were maintained in MRS broth (BBL, USA) at 37℃, and subcultured every 18 h or 24 h. A stock of all identified isolates was stored in bacterial storing vials (PROTECT, England) at −70℃ for use later.

Preparation of intestinal section for scanning electron microscopy

Intestinal sections for scanning electron microscopy (SEM) were prepared using a modified method of Salanitro et al. (1978). Three 21-day-old chickens were sacrificed. When their body cavities were exposed, cotton pads soaked in cold (4℃) fixative [(2% glutaraldehyde in 0.1M phosphate buffer (pH 7.0)] were immediately placed over the exposed intestinal tracts. The intestinal tract was divided by ligation into duodenal, jeju-ileal and cecal sections, and cold fixative was injected to fill up the lumen of each section. After 10 min, 0.5 cm^2 of tissues were cut from each intestinal section and pinned, with the epithelial side exposed, onto pieces of dental wax. The sections were placed in cold fixative for 2 h and transferred to fresh fixative for 24 h. They were then washed three times with cold phosphate buffer, postfixed with 2% OsO_4 in phosphate buffer (pH7.0) for 2 h, and washed again with buffer to remove the fixative. Dehydration was carried out with ascending concentrations of acetone: 35%, 50%, 75%, 95% (10 min each) and 100% (15 min, three times). The samples were dried for 20 min in a critical-point drier (HCP-2, HITACHI, Japan), mounted on stubs, and coated with gold using a sputter coater (Polaron E5,100, UK). Coated specimens were examined with a scanning electron microscope (JEOL, JSM6400).

Statistical analysis

Effects of media and incubation time on the total colony counts were analyzed by two-factor analysis of variance followed by least significant difference using the SAS program (SAS, 1985).

RESULTS

Comparison of media and incubation period for intestinal bacteria

Since there was no significant interaction between two factors (i.e., between media and incubation period), the results were analyzed to determine the main effects of each factor. The numbers of bacterial colonies from the duodenal, jeju-ileal and cecal contents cultured in MOD-SD and M98-5 media were not significantly different (table 2). However, the numbers of bacterial colonies from all the three parts of the intestine were found to be significantly higher ($p < 0.05$) in FM98-5 medium than in either MOD-SD or M98-5 medium. With regard to the incubation time, the bacterial colony counts from the contents of the three parts of the intestine on each

Table 2. Comparison of bacterial colony counts from duodenal, jeju-ileal and cecal contents on M98-5, FM98-5 and MOD-SD media at different incubation times

Incubation (days)	Log CFU/(g · DM)*			Statistical significance between media†‡
	MOD-SD	M98-5	FM98-5	
From duodenal contents				
2	1.28 ± 0.24[a]	0.82 ± 0.43[a]	4.10 ± 0.82[a]	S (MOD-SD or M98-5 vs. FM98-5)
6	2.30 ± 0.53[b]	1.83 ± 0.57[b]	5.90 ± 0.78[b]	S (MOD-SD or M98-5 vs. FM98-5)
10	2.73 ± 0.64[b]	2.36 ± 0.71[b]	6.70 ± 0.75[b]	S (MOD-SD or M98-5 vs. FM98-5)
From jeju-ileal contents				
2	2.68 ± 0.64[a]	2.48 ± 0.63[a]	4.75 ± 0.45[a]	S (MOD-SD or M98-5 vs. FM98-5)
6	3.65 ± 0.58[b]	3.48 ± 0.60[b]	6.82 ± 0.71[b]	S (MOD-SD or M98-5 vs. FM98-5)
10	4.03 ± 0.73[b]	4.36 ± 0.94[b]	7.18 ± 0.53[b]	S (MOD-SD or M98-5 vs. FM98-5)
From cecal contents				
2	3.02 ± 0.58[a]	2.78 ± 0.74[a]	5.67 ± 0.63[a]	S (MOD-SD or M98-5 vs. FM98-5)
6	5.30 ± 0.63[b]	5.35 ± 0.75[b]	8.78 ± 1.00[b]	S (MOD-SD or M98-5 vs. FM98-5)
10	5.77 ± 0.71[b]	5.81 ± 0.89[b]	9.70 ± 0.63[b]	S (MOD-SD or M98-5 vs. FM98-5)

CFU, colony forming units;
MOD-SD, Scott and Dehority medium; FM98-5, M98-5 + fecal extract;
* Values presented were means of counts from 3 chickens, each with a duplicate;
[ab] Within each medium, comparison of significance is made among incubation times; Means not followed by the same letter in the same column are significantly different ($p < 0.05$);
† At each medium, comparison of significance is made between media;
‡ S, counts are significantly different ($p < 0.05$);

medium at 6 days of incubation were not significantly different from those at 10 days of incubation, but they were significantly higher ($p < 0.05$) than those at 2 days of incubation (table 2).

Table 3 shows the percentage of recoveries of cecal anaerobes from the three media. The percentage of recovery of cecal anaerobes from M98-5 medium and MOD-SD medium was similar, but was much lower than that from FM98-5 medium. However, it was not possible to calculate the percentage of recovery of anaerobes present in the duodenum and jeju-ileum because the number of bacteria in most of these samples was too low. Furthermore, the presence of debris in the samples interfered with accurate microscopic enumeration of the bacteria.

Table 3. Percentage recovery of anaerobes from cecal samples in three different media

Incubation time (days)	Recovery of bacteria (%)*		
	MOD-SD	M98-5	FM98-5
2	15.10	13.90	28.35
6	26.50	26.75	43.90
10	28.85	29.05	48.50

MOD-SD, Scott and Dehority medium; FM98-5, M98-5 + fecal extract;
* Percentage of recovery was calculated as follow: colony counts/total microscopic counts.

Bacterial populations in the duodenum, jeju-ileum and cecum

Table 4 shows the anaerobic and aerobic bacterial populations (enumerated as Log CFU / g DM) in the small intestine and cecum. In all the three regions of the intestinal tract, the population of the anaerobic bacteria was significantly ($p<0.05$) higher than that of the aerobic bacteria. Of the three intestinal sections, the cecum had the largest anaerobic and aerobic bacterial populations.

Table 4. Bacterial populations in the intestinal tract of chicken

Intestinal region	Log CFU/g DM	
	Anaerobe*	Aerobe†
Duodenum	6.14 ± 0.25^a	5.30 ± 0.23^b
Jeju-ileum	8.61 ± 0.32^a	7.20 ± 0.13^b
Cecum	10.82 ± 0.06^a	8.54 ± 0.17^b

CFU, colony forming units;
Means not followed by the same letters in the same row are significantly different ($p < 0.05$);
* Roll tube counts in FM98-5 medium;
† Plate counts in BHIA medium.

Isolation and identification of intestinal bacterial strains

Colonies isolated from the duodenum, jeju-ileum and cecum of 5 birds (21-day-old) were grouped and tentatively classified on the basis of morphology, Gram stain, pH changes during growth with glucose as a substrate and products formed from glucose fermentation.

Both rods and cocci were isolated from the duodenum and jeju-ileum (table 5). The types of bacteria isolated included *Lactobacillus, Staphylococcus, Streptococcus* and anaerobic coccus. Strains of catalase-negative, Gram-positive rods that grew anaerobically on MRS agar were tentatively identified as *Lactobacillus* (Gilliland et al., 1975). These isolates grew well on PYG medium and produced large amount of lactic acid.

Table 5. Presumptive identification features of bacteria isolated from the duodenum and jeju-ileum of chicken*†

Morphology	Gram stain	Aerobic growth	pH	Fermentation products	Tentative identification
Rod	+	+	3.75 – 4.40	L, a	*Lactobacillus*
Rod	+	–	4.61	L, a, s	*Lactobacillus*
Rod	–	+	NT	L, a, s, (F)	*E. coli*
Rod	–	–	4.42 – 5.02	L, a, p	*Fusobacterium*
Coccus	+	+	NT	L, (F, a) L, (f, a)	*Staphylococcus or Streptococcus*
Coccus	+	–	3.94 – 4.64	L, a, p, s	Anaerobic coccus
Coccus	–	–	4.64 – 6.02	L, a	unknown

* Data compiled from 180 bacterial strains isolated from 3 chickens;
† NT, Not tested; +, positive reaction for 90-100% strains; –, negative reaction for 90-100% strains; A, a (acetic); P, p (propionic); B, b (butyric); L, l (lactic); S, s (succinic); upper case letters refer to large amount of production, and lower case letters refer to small amount of production; products in parenthesis are variable and are produced by a few strains.

Gram-negative bacteria, which grew as pink colonies on plates of MacConkey medium (Difco), were identified as *E. coli*. The Gram-positive, catalase-negative cocci, which produced lactic and acetic acids in PYG medium, were presumptively identified as *Streptococcus*. Gram-positive, catalase-positive cocci were grouped into *Staphylococcus*. There were some anaerobic, Gram-negative cocci which could not be identified with certainty.

Anaerobic bacteria found in the cecum seemed to be more diversed than those in the duodenum and jeju-ileum (table 6). Besides obligate anaerobic bacteria, facultative anaerobic bacteria were also isolated from the cecum, but their numbers were low. The cecal facultative anaerobic bacteria included *Streptococcus* (*Staphylococcus*), *Lactobacillus*, and *E. coli*.

Anaerobic Gram-negative cocci, producing large amounts of acetic and propionic acids, could not be identified with certainty, but they resembled *Veillonella* in the rumen (Kudo et al., 1979). Gram-positive cocci arranged in pairs and chains were tentatively identified as *Peptostreptococcus* although they had different acid products from glucose fermentation.

Table 6. Presumptive identification features of bacteria isolated from the cecum of chicken*†

Morphology	Gram reaction	Aerobic growth	Final pH on glucose	Fermentation products	Tentative identification
Coccus	+	+	NT	NT	*Streptococcus or Staphylococcus*
	+	−	5.40 − 5.64	L, A	*Peptococcus*
	+	−	5.00 − 5.79	A, (l, p, b, v)	*Peptostreptococcus*
	+	−	5.01 − 5.33	S, L	*Ruminococcus*
	−	−	5.62 − 5.76	P, A	*Veilonella*
Rod	+	+	3.95 − 4.68	L, a	Aerobic *Lactobacillus*
	+	−	3.78 − 5.03	L, a	Anaerobic *Lactobacillus*
	+	−	4.99	L, A	*Eubacterium*
	+	−	4.69 − 4.89	P, A, (l, s)	*Propionibacterium*
	+	−	5.29 − 6.03	B, (a, p, iv)	*Clostridium*
	−	+	4.90 − 5.20	L, A. s	*Escherichia coli*
	−	−	5.04 − 5.86	A, l, p, b, iv	*Bacteriodes*
	−	−	5.29 − 6.03	B, a	*Fusobacterium*
	−	−		A, (l, s)	*Bacteroides*
	−	−		a, s	*Bacteroides*

* Data compiled from 120 strains isolated from 3 chickens;

† NT, not tested; +, positive reaction for 90-100% strains; −, negative reaction for 90-100% strains; A, a (acetic); P, p (propionic); B. b (butyric); V. v (valeric); IV, iv (iso-valeric); L, l (lactic); S, s (Succinic); upper case letters refer to large amount of production, and lower case letters refer to small amount of production; products in parenthesis are variable and are produced by a few strains.

One of the largest groups of anaerobes isolated was Gram-positive, irregular rods. Most of the strains were obligate anaerobes, but a few were also facultative. These were identified as *Lactobacillus, Eubacterium*, and *Propionibacterium* according to the types of acids produced. Those that were pleomorphic, nonmotile and spore-forming, were similar to *Clostridium*.

No attempt was made to identify the 300 isolated strains to species level based on the above-mentioned characteristics since this study was conducted to observe the general profile of intestinal microflora in chicken.

Distribution of bacteria in the intestinal tract

It can be seen in table 7 that the majority (68.5% to 77.1%) of bacteria isolated were rod-shaped and most of them were Gram-positive. Bacteria in the small intestine (i.e. duodenum and jeju-ileum) were mainly (71.0-75.0%) facultative anaerobes whereas those in the cecum were mostly (94.2%) obligate anaerobes (table 7). In the small intestine, *Streptococcus, Lactobacillus* and *E. coli* were the major genera, comprising 96.5% of the bacteria in the duodenum, and 87.5% in the jeju-ileum. *Staphylococcus* or *Fusobacterium* were also isolated from the small

Table 7. Distribution of intestinal bacteria in chicken

Bacterial group*	Percentage of total isolates in each intestinal section		
	Duodenum	Jeju-ileum	Cecum
Streptococcus	20.0	18.8	2.5
Staphylococcus	1.0	1.5	–
Lactobacillus	60.0	51.7	1.3
E. coli	16.5	17.0	2.0
Anaerobic coccus	2.5	5.8	20.4
Eubacterium	–	–	21.2
Propionibacterium	–	–	2.0
Clostridium	–	–	8.0
Fusobacterium	–	5.2	12.0
Bacteroides	–	–	30.6
Facultative anaerobe (%)	75.0	71.0	5.8
Anaerobe (%)	25.0	29.0	94.2

* Bacterial groups listed in Table 5 and 6;
–, No strains isolated.

Table 8. Catalase reaction, growth and carbohydrate fermentation of *Lactobacillus spp*

Tentative Identification	*L. acidophilus*	*L. fermentum*	*L. brevis*	*L. delbrueckii*	*L. Lactis*	*L. crispatus*	*L. salivarus*	*L. plantarum*
Gas (catalase reaction)	–	–	–	–	–	–	–	–
Growth at 15℃	–	–	–	–	–	–	–	–
Growth at 45℃	(+)	+	+	+	+	+	+	+
Amidon	(+)	–	–	–	–	+	–	–
Amygdalin	+	–	–	–	–	+	–	+
D-Arabinose	–	–	–	–	–	–	–	–
L-Arabinose	–	–	+	–	+	–	–	(+)
Cellobiose	+	–	–	–	+	+	–	+
D-Fructose	+	(+)	+	+	+	+	+	+
Galactose	(–)	+	(–)	+	+	+	+	+
D-Glucose	+	+	+	+	+	+	+	+
Gluconate	–	+	+	–	+	–	(–)	(–)
Lactose	W	+	(–)	–	+	+	+	+
Maltose	+	+	+	–	+	+	+	+
Mannitol	–	–	W	–	W	–	+	+
D-Mannose	+	–	–	+	+	+	+	+
Melezitose	–	–	–	–	–	–	–	–
Melibiose	+	–	+	(+)	+	–	+	–
D-Raffinose	+	+	(–)	–	+	+	+	(–)
Rhamnose	–	–	–	–	–	–	+	–
Ribose	–	+	+	W	+	–	–	+
Salicine	+	–	W	–	–	+	+	+
Sorbitol	–	–	–	–	–	–	+	+
Sucrose	NT	NT	NT	NT	NT	NT	NT	NT
Trehalose	(–)	–	–	–	–	(+)	+	(+)
D-Xylose	–	(–)	(+)	–	–	–	–	–
L-Xylose	–	–	–	–	–	–	–	–
Esculin	+	–	(+)	–	W	+	–	+

+, positive reaction; (+), most positive; –, negative; (–), most negative; W, weak or slow reaction; NT, not tested.

intestine.

In the cecum, *Bacteroides*, *Eubacterium* and anaerobic cocci were the predominant anaerobes accounting for 30.6%, 21.2% and 20.4% of the total bacterial isolates, respectively. Other anaerobic bacteria which were recovered in smaller numbers included *Propionibacterium*, *Clostridium*, *Fusobacterium* and *Lactobacillus*.

Isolation and identification of intestinal *Lactobacillus*

A total of 56 *Lactobacillus* isolates were obtained from the jeju-ileum and cecum. They were tentatively identified by Gram stain, catalase reaction, growth at 15℃ and 45℃ and carbohydrate fermentation using the API Kit system (table 8). Of these isolates, *Lactobacillus acidophilus*, *L. fermentum*, and *L. brevis* were the main species, comprising 23.2%, 16.1% and 19.6% of the total isolates, respectively (table 9). *L. delbrueckii* and *L. lactis* were isolated from both the jeju-ileum and cecum, but *L. crispatus*, *L. plantarum* and *L. salivarus* were only found in the jeju-ileum, but not in the cecum.

Table 9. Distribution of *Lactobacillus* spp. in the chicken intestine

Lactobacillus sp	Number of isolates identified			Percentage
	Jeju-ileum	Cecum	Total	
Heterofermentative	11	9	20	35.7
L. fermentum	3	6	9	16.1
L. brevis	8	3	11	19.6
Homofermentative	18	9	27	48.2
L. acidophilus	8	5	13	23.2
L. delbrueckii	2	2	4	7.1
L. lactis	2	2	4	7.1
L. crispatus	3	0	3	5.4
L. salivarus	2	0	2	3.6
L. plantarum	1	0	1	1.8
Unidentified	4	5	9	16.1
Total	33	23	56	100.0

Scanning electron microscopy (SEM) of the intestinal epithelia

SEM was used to observe the bacterial populations inhabiting the epithelial surface of the intestinal tract of chicken. Very few or no bacteria were observed on the surface of the duodenal mucosa. This may be due to the low number of bacteria on the epithelial surface of the duodenum and some of these might be dislodged when the specimens were processed for SEM. On the other hand, more bacteria were found on the epithelium of jeju-ileum. They were mostly thick, long or short rod-shaped bacteria (figure 1). The largest population of bacteria was observed on the epithelial surface of the cecum (figure 2). Dense colonies of mixed rods and cocci and a few spirillum covered the epithelial surface of the cecum (figure 2).

Figure 1. Scanning electron micrograph of the jeju-ileal epithelial surface of a 21-day-old chicken showing thick rod-shaped bacteria, singlely or in chanis.
Bar=2.0 μm.

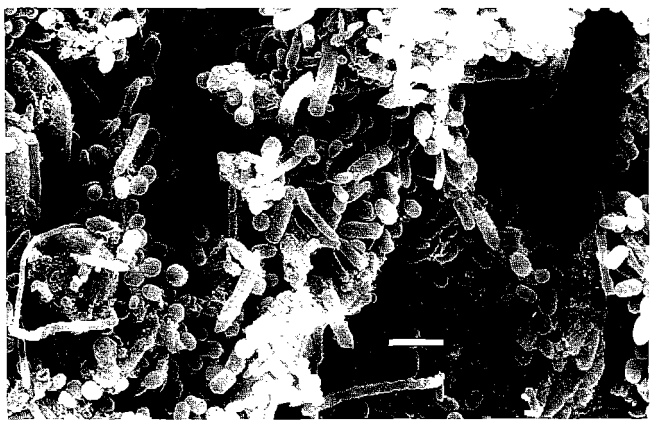

Figure 2. Scanning electron micrograph of the cecal epithelial surface of a 21-day-old chicken showing dense population of bacteria comprising mixed rods, cocci and spirilum covering the epithelial surface.
Bar=2.0 μm.

DISCUSSION

The results of the present study showed that generally, the addition of 10% fecal extract in the culture medium (FM98-5 medium) increased the bacterial colony counts from chicken intestinal samples. This result agrees with the findings of Barnes and Impey (1970) in which the addition of chicken fecal extract to the medium (M10) was necessary for the isolation of many fastidious

anaerobes from chicken cecum. Since FM98-5 medium was found to be the most suitable medium for the growth of intestinal bacteria of chicken, it was used for the isolation of anaerobic bacteria in chicken intestine in subsequent studies.

Of the three regions of the alimentary tract, i. e., duodenum, jeju-ileum and cecum, the duodenum had the lowest population of bacteria and the cecum the highest. The same pattern was also observed with SEM examination of the epithelial surface of the intestinal tract. Salanitro et al. (1978) reported similar finding that the epithelia of the duodenum and parts of the ileum in chicks were sparsely populated by bacteria.

The major types of bacteria found in the duodenum and jeju-ileum of chicken in this study were *Lactobacillus*, *Streptococcus* and *E. coli*. *Staphylococcus*, anaerobic cocci and *Fusobacterium* occurred in lesser numbers. These results are similar to those of Barnes et al. (1972) and Smith (1965) who reported that *Streptococcus*, lactobacilli and *E. coli* were the predominant bacteria in the duodenum and ileum of 2-week-old chicks. Similarly, Salanitro et al. (1978) found that 60 to 90% of the small intestinal bacteria in 2-week-old chicks was represented by these three bacterial groups. They also reported that the small intestine was inhabited by diverse anaerobic bacterial types which included anaerobic cocci, *Eubacterium*, *Propionibacterium*, *Clostridium*, *Gemmiger* and *Fusobacterium*, but these occurred in lesser numbers.

It is not surprising to find that anaerobes comprised nearly the entire microbial population of the cecum. Ten groups of bacteria, namely, *Streptococcus*, *Staphylococcus*, *Lactobacillus*, *E. coli*, anaerobic coccus, *Eubacterium*, *Propionibacterium*, *Clostridium*, *Fusobacterium* and *Bacteroides* were identified. This result agrees with the finding of Salanitro et al. (1974b; 1978) and Mead (1989) who reported that obligate anaerobes (anaerobic cocci, *Eubacterium*, *Clostridium*, *Gemmiger*, *Fusobacterium*, and *Bacteroides*) made up nearly the entire microbial population of the cecum.

The main *Lactobacillus* species in the jeju-ileum or cecum of chicken in the present study were identified as *L. acidophilus*, *L. fermentum* and *L. brevis*. Species of *L. delbrueckii*, *L. lactis*, *L. crispatus* and *L. plantarum* were also found in either the jeju-ileum or cecum or both. These results agree in part with Mitsouka (1969) who showed that *L. acidophilus*, *L. salivarius* and *L. fermentum* were the most common species in the chicken intestine. The results are also similar with the finding of Sarra et al. (1985) who found that homofermentative lactobacilli were dominant in all sections of the intestine. Most reports on fowl lactic acid bacteria are on isolates obtained from the lower parts of the alimentary tract and from the crop (Barnes, 1979). Investigations on lactobacilli in other intestinal areas are lacking and more studies need to be carried out on them.

CONCLUSION

The results of this study showed that (i) FM98-5 medium was the most suitable medium for isolation of anaerobic bacteria from chicken intestine which indicated that supplementation of fecal extract in the medium increased the number of bacterial colonies growing on it; (ii) the types of bacteria isolated from the duodenum and jeju-ileum included *Lactobacillus*, *Staphylococcus*, *Streptococcus* and anaerobic coccus; (iii) *Bacteroides*, *Eubacterium* and anaerobic cocci were the predominant anaerobes in the cecum; and (iv) of those *Lactobacillus* spp. isolated, *L. acidophilus*, *L. fermentum*, and *L. brevis* were the main species.

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