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Aerobic and Facultatively Anaerobic Bacteria Associated with the Gut of Canada Geese (*Branta canadensis*) and Whistling Swans (*Cygnus columbianus columbianus*)

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Aerobic and facultatively anaerobic bacteria from the intestinal tracts of swans and geese were isolated and characterized as part of a larger study of the microbiological effects of migratory waterfowl on water quality. A total of 356 isolates were identified by using rapid identification methods and classified by using numerical taxonomy. A diverse population of bacteria was recovered from the waterfowl, and representative strains could be classified into 21 phena. The majority of the aerobic, heterotrophic bacteria found in the gut of the waterfowl were species of *Enterobacteriaceae*, *Streptococcus*, *Lactobacillus*, and *Bacillus*. Unfortunately, the birds that were examined did not harbor significant numbers of any waterfowl-specific bacterial species. Thus, it may not be possible to assess microbiological impact of migratory waterfowl by using an "indicator" species since avian fecal pollution could not be distinguished from animal and human fecal pollution.

Although the intestinal flora of ruminating animals, humans, and other mammals has been relatively well documented, less is known about the bacteria associated with the gut of fowl (3, 6, 12, 16, 23, 31, 33, 43-45). In fact, the intestinal bacteria of healthy, normal avian species found in the wild, notably waterfowl, have rarely been examined (35).

Recently, we reported that whistling swans and Canada geese may contribute sufficient quantities of enteric organisms into an estuary to elevate microbiological water quality index and that wild waterfowl, in general, maintain larger numbers of aerobic bacteria in the gut than captive waterfowl (24). The purpose of the study reported here was to characterize this aspect of the gut flora of waterfowl, both to determine the species composition of these bacterial populations found in the gut of wild and captive swans and geese and to seek a microbial indicator of wild avian fecal contamination.

MATERIALS AND METHODS

Isolation and maintenance of the aerobic heterotrophic bacterial strains. The sampling methods used to collect fresh fecal material from wild and captive Canada geese (*Branta canadensis*) and whistling swans (*Cygnus columbianus columbianus*) have been described elsewhere (24). Colonies were randomly picked from tryptic soy agar (TSA; Difco Laboratories, Detroit, Mich.) plates, used in the enumeration of the gut flora of the animals, after incubation for 72 h at 37°C. The isolates were purified by successive streaking on TSA and stored on TSA slopes under mineral oil at room temperature (20 to 25°C). Working stock cultures were transferred at 6- to 8-week intervals.

Characterization of the strains. On the basis of glucose metabolism, recorded after incubation of the inoculated test medium for 7 days at 37°C, each of 356 pure cultures was identified as a glucose oxidizer or fermenter (22). Gram-negative, oxidase-negative, aerogenic fermentative strains, presumptively identified as *Enterobacteriaceae*, were confirmed by using the API-20E rapid identification system (Analytab Products, Plainview, NY). Strains not grouped as *Enterobacteriaceae* were further analyzed by numerical taxonomy. A total of 255 strains, for which 112 unit characters were recorded, were included in the analysis (see Table 1).

Unless otherwise specified, all media were inoculated with 18- to 24-h cultures grown on TSA and were incubated at 37°C for 7 days. Details of the methods used to characterize the isolates have been described (7, 18), except for the following modifications. TSA was used as the basal medium for detection of hemolytic activity, NaCl tolerance, and growth at selected temperatures. Sensitivity to the vibriostatic agent 0/129 (pteridine) was measured on TSA by placing several crystals of the 0/129 compound on freshly seeded plates.

Computer analysis of the taxonomic data. The data, comprising 112 unit characters scored for the 255 strains demonstrating oxidative metabolism of glucose

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or lack of glucose utilization, were computed by using the simple matching coefficient (S_{SM}; 48, 49), which includes positive and negative matches, and the Jaccard coefficient (S_J; 46), which excludes negative matches. Clustering was by unweighted average linkage (48, 49), from which sorted similarity matrices and dendrograms were constructed. The programs used included the UMDTAXON package of programs available on the University of Maryland UNIVAC 1108 computer.

RESULTS

The majority (64%) of the 101 strains that were fermentative were classified as *Escherichia coli* according to the API-20E method for rapid identification. The remaining 36% of the fermenters were distributed among 13 taxa: *Enterobacter agglomerans* (7%); *Klebsiella pneumoniae* (6%); *Enterobacter cloacae* (6%); *Citrobacter freundii* (3%); *Hafnia* (3%); *Klebsiella ozaenae* (2%); *Klebsiella rhinoscleromatis* (2%); and *Proteus morganii* (1%). Strains originally isolated from media used for enrichment for *Salmonella* (24) were: *Pseudomonas fluorescens* (2%); *Aeromonas hydrophila* (2%); *Acinetobacter* spp. (1%); *Alcaligenes* spp. (1%); and *Pseudomonas maltophilia* (1%). It is important to note that less than 20% of the total set of 356 isolates, on initial screening, proved to be *E. coli* type I and only 38% were representatives of the *Enterobacteriaceae*.

Two hundred and forty-three strains, 95% of those subjected to the numerical taxonomy analysis, constituted 21 clusters as defined from results of computations using the S_J and S_{SM} coefficients. A simplified dendrogram, based on results obtained with the S_J coefficient, is shown in Fig. 1. The clusters were identified by using the diagnostic tables of Cowan (8, 9), diagnostic keys provided in *Bergey's Manual of Determinative Bacteriology* (5), and specialist keys of Gordon et al. (19) and others, as cited below.

Phenon 1, the largest cluster, comprising 54 strains, was classified as species of group D *Streptococcus* (10). Characteristically, the strains were gram-positive cocci that formed short chains and utilized glucose oxidatively/fermentatively. Catalase was produced, and a majority of the strains also produced arginine dihydrolase, degraded esculin and gelatin, and grew at 42°C and in the presence of 7.5% (wt/vol) NaCl. The results were consistent with those obtained for group D *Streptococcus*, i.e., *S. faecalis*, *S. faecium*, and *S. avium*.

Phenon 2, with 17 isolates, was identified as *Arthrobacter* spp. (26) based on distinctive micromorphology (i.e., short rods or cocci, irregular rods, and large irregular cocci), catalase production, oxidase production, and alkaline reaction in the oxidation-fermentation medium. The organisms were motile, gram-negative coccobacilli that did not produce arginine dihydrolase or hydrolyze starch or gelatin. Although generally described as "readily decolorized," gram-positive cells (26), a number of species, e.g., *A. atrocyaneus*, *A. globiformis*, and *A. pascens*, are gram negative in young cultures (4). The pigmentation of the colonies, for a number of the strains, was similar to that described by Jones (25) for coryneforms and related bacteria, i.e., *Arthrobacter*.

Phenon 3 consisted of four strains of gram-positive, nonsporeforming, nonmotile, irregularly shaped rods. These failed to hemolyze blood or reduce nitrate, but did produce an alkaline reaction in the oxidation-fermentation medium. Such isolates were provisionally classified as "coryneform" bacteria, in accordance with descriptions provided by Rogosa et al. (41). The temperature range of growth for the strains was high, i.e., in the range of 37 to 42°C.

The four strains in phenon 4 were presumptively identified as belonging to the genus *Flavobacterium* on the basis of yellow pigment, oxidative metabolism, absence of motility, and production of oxidase. Furthermore, these rods displayed the general characteristics of *Flavobacterium meningosepticum*. In particular, the isolates grew in the presence of 7.5% (wt/vol) NaCl and hemolyzed blood but did not hydrolyze gelatin or casein and failed to reduce nitrate. Two of the strains were capable of utilizing citrate as sole carbon source. These isolates, however, grew at 42°C (not considered to be characteristic of *F. meningosepticum* [50]), suggesting further analysis of these strains to be warranted before their identification can be confirmed.

Phenon 5 contained 34 strains, presumptively identified as *Lactobacillus* spp. on the basis of micromorphology, nonmotility, fermentative metabolism, absence of catalase, and inability to degrade casein and gelatin or to reduce nitrate (40). The micromorphology, temperature range for growth, anaerogenic glucose fermentation, and hydrolysis of esculin strongly indicated that *L. coryniformis* was the principal species in the phenon.

The three strains of phenon 6 were identified as *Kurthia* spp. from descriptions of Keddie and Rogosa (28). Identification was based on micromorphology, i.e., motile, pleomorphic, gram-positive rods occurring singly and in chains, as well as on their oxidative metabolism and catalase, but not oxidase, production. The strains of the phenon were unable to reduce nitrate, hemolyze blood, or decarboxylate arginine. Furthermore, the isolates possessed characteristics typical of *K. zopfii*, i.e., inability to degrade starch or esculin, lack of urease, presence of yellow pigment,

TABLE 1. *Summary of characteristics*

Phenon	No. of strains	Colony			Diffusible pigment	Fluorescent pigment	Gram reaction	Cell morphology[c]	Spore location[d]	Motility	Arginine dihydrolase	Catalase	Fermentative	Oxidative	Alkali production	Oxidase	Nitrate reduction	Nitrite reduction	Degradation of:						
		Convex	Opaque	Color[b]															Blood	Casein	Esculin	Gelatin	Starch	Tyrosine	Urea
1	54	+	D	–	–	–	+	C	–	–	D	D	D	D	D	D	D	–	D	D	D	D	–	–	–
2	17	+	–	–	–	–	D	R	–	D	–	+	–	–	+	+	D	–	–	–	–	–	–	D	–
3	4	+	–	–	–	–	+	R	–	–	–	+	–	–	D	D	–	–	–	–	–	–	–	D	–
4	4	+	–	Y	–	–	–	R	–	–	D	+	–	D	–	+	–	–	D	+	–	+	–	D	–
5	34	+	–	–	–	–	+	R	–	–	D	–	+	+	–	–	–	–	D	–	+	–	–	–	D
6	3	–	–	Y	–	–	+	R	–	+	–	+	–	–	+	–	–	–	–	–	–	D	–	–	–
7a	19	D	D	–	–	–	+	R	C	+	–	+	–	+	–	D	–	–	+	+	+	+	–	–	–
7b	5	+	D	Y	–	–	+	R	–	+	–	+	–	+	–	D	–	–	D	+	+	+	–	–	–
8	10	+	D	O	–	–	+	R	–	D	–	–	–	D	–	+	–	–	–	+	+	+	+	D	–
9	16	+	+	P	–	–	+	R	ST	+	–	+	–	+	–	–	–	–	–	+	+	+	+	+	–
10	2	+	–	–	–	–	+	R	–	+	–	+	–	–	–	+	D	D	+	+	–	+	+	D	–
11	14	+	+	–	–	–	+	R	ST	+	+	+	–	+	–	+	+	–	+	+	+	+	+	+	–
12	3	+	D	–	–	–	+	BR	–	–	–	+	–	D	–	–	+	–	–	D	D	+	+	–	–
13	31	+	–	–	–	–	–	R	–	+	D	+	+	+	–	–	+	–	D	–	+	–	–	–	–
14	3	+	–	–	–	–	–	R	–	–	–	+	+	+	–	–	+	–	D	–	+	–	–	–	+
15	3	+	+	–	–	–	–	R	–	+	–	+	+	+	–	–	+	–	D	–	+	–	–	–	+
16	3	+	–	–	–	–	–	R	–	+	–	+	–	–	+	–	–	+	–	–	+	–	–	–	D
17	5	+	–	–	O	D	–	R	–	D	+	+	–	+	–	+	–	–	–	–	–	–	–	+	–
18	7	+	–	–	–	–	–	R	–	D	–	+	–	D	–	D	–	–	D	–	+	+	+	–	–
19	2	+	–	V	–	–	–	R	–	D	–	+	–	–	D	+	+	–	+	+	+	+	D	+	–
20	2	D	–	–	–	–	–	R	–	–	–	+	–	–	+	–	–	–	–	–	–	–	–	D	–
21	2	+	–	Y	–	–	D	R	–	–	–	+	–	–	D	–	+	–	+	D	–	+	D	+	–

[a] +, ≥80% positive reaction; –, ≤20% positive reaction; D, 21 to 79% positive reaction. Growth at 37°C and in 2.5% NaCl were excluded from the table since they proved to be non-differentiating characters.

[b] Y, Yellow; O, orange; P, pink; V, violet.

[c] R, Rod; C, coccus; B, branched.

[d] C, Central; ST, subterminal.

and growth at 42°C (15). The single deviation noted from the species description for *K. zopfii* was the inability to hydrolyze gelatin.

Phenon 7 could be subdivided into two subphena, 7a and 7b, at the 85 to 90% S level. The subphena were presumptively identified as biotypes of *Bacillus pumilus*, according to descriptions of Gordon et al. (19) and Gibson and Gordon (17). Members of this taxon are characterized as motile, catalase-positive, gram-positive bacilli. The strains utilized citrate, hemolyzed blood, and hydrolyzed casein, esculin, and gelatin, but did not reduce nitrate. Phenon 7a, containing 19 strains, was primarily distinguished from the five strains of phenon 7b by a variable gram reaction, presence of spores, lack of pigmentation, and ability to use arabinose, but not histidine, as a sole carbon source. A yellowish pigment, noted by Gibson and Gordon (17) as a distinctive characteristic of *B. pumilus*, was produced by the isolates of 7b but not by those of 7a.

Phenon 8 contained 10 orange-pigmented, oxidative, oxidase-positive, catalase-negative, motile, gram-positive rods possessing characteristics intermediate between those of *Lactobacillus* and *Bacillus* (40). As a consequence of the difficulties met in identifying strains of this phenon, it is concluded to be an unidentified taxon.

Phena 9 and 10, containing 16 and 2 isolates, respectively, were presumptively classified as *Bacillus megaterium*, according to descriptions of Gordon et al. (19). Classification and identification are based on micromorphology, catalase production, ability to degrade casein and starch, utilization of sodium citrate, and ability to grow at temperatures ranging from 10 to 42°C and in the presence of 7.5% (wt/vol) NaCl. Despite these characteristics being typical of *B. megaterium*, the organisms were recovered in two distinct clusters. Hence, both groups are tentatively considered to be biotypes of the same species, until further studies can be done. The nitrate-reducing strains grouped together as phenon 10. It is possible that phena 9 and 10

of phena isolated from waterfowl[a]

NaCl				Growth at:			Utilization of:															
5%, wt/vol	7.5%, wt/vol	10%, wt/vol	0/129 Sensitivity	5°C	10°C	42°C	Adonitol	Alanine	Arabinose	Cellobiose	Ethanol	Fructose	Galactose	Glucose	Histidine-HCl	Lactose	Maltose	Sodium citrate	Sodium malonate	Raffinose	Ribose	Trehalose	Xylose
+	+	D	−	−	+	+	−	−	−	−	−	D	−	−	−	−	−	−	−	−	D	D	−
D	D	D	D	D	+	−	−	−	−	−	−	−	−	D	−	−	−	−	−	−	−	−	−
D	D	D	D	−	−	+	−	−	−	−	−	−	−	D	−	−	−	−	−	−	−	−	−
D	D	−	−	+	+	+	−	−	−	−	−	−	−	−	−	−	D	D	−	−	−	−	−
D	−	−	+	D	+	D	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
+	D	−	−	−	+	+	−	+	−	−	−	D	−	−	−	−	−	−	−	−	D	−	−
+	+	+	+	−	+	+	−	+	+	+	−	+	D	+	−	D	D	+	−	D	+	+	+
+	+	+	D	−	+	+	−	+	−	+	−	+	D	+	D	−	D	+	−	+	+	+	+
+	+	+	−	−	+	+	−	+	−	D	−	+	D	+	D	−	+	D	−	−	+	+	−
+	+	+	D	−	+	+	−	+	+	+	D	+	+	+	+	+	+	+	−	+	+	+	+
+	+	+	+	−	+	+	−	D	D	+	D	+	−	+	+	−	+	+	+	+	+	+	−
+	+	−	−	−	+	+	−	+	−	+	−	+	−	+	+	−	+	+	−	−	+	+	D
+	−	−	−	−	−	+	−	+	+	+	−	+	+	+	−	D	+	+	−	−	+	+	+
D	D	−	−	D	+	D	−	+	+	D	−	+	+	+	−	D	+	D	−	+	+	+	+
+	+	−	−	−	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
+	+	−	−	−	+	+	−	+	+	D	−	+	D	+	D	+	D	+	D	−	+	+	+
+	+	−	−	−	+	+	+	+	+	+	+	+	+	+	+	D	+	+	+	+	+	+	+
−	−	−	−	+	+	−	−	+	+	−	D	+	D	+	+	D	−	+	D	−	+	D	D
+	−	−	D	−	+	+	−	−	+	+	−	+	+	+	−	+	+	−	−	+	+	+	+
+	−	−	−	−	+	+	−	D	+	−	−	D	D	+	−	D	D	D	−	−	−	−	−
−	−	−	−	+	+	−	+	+	−	−	+	D	+	D	−	+	+	+	D	+	−	−	−
+	+	D	−	−	+	−	D	+	+	+	+	+	+	+	D	+	+	−	−	−	+	D	D

exemplify a case of "two merging aggregates of strains," as described by Gordon et al. (19). Phenon 11, comprising a total of 14 strains, was classified as *Bacillus cereus* (17, 19).

The three strains in phenon 12 were identified as streptomycetes, largely on the basis of distinctive micromorphology, i.e., stable branching, sporing mycelia, and other characteristics listed in Table 1, according to descriptions of Kuster (29), Gottlieb (20), and Pridham and Tresner (37, 38).

Phenon 13 contained 31 strains presumptively identified as members of the *Enterobacteriaceae*, according to the diagnostic tables of Cowan (8, 9), but they could not be identified to species because of atypical characteristics intermediate between those of *E. coli* and *H. alvei*.

Phenon 14 contained three strains presumptively identified as *Klebsiella pneumoniae*, on the basis of acid and gas production from carbohydrates, absence of motility, production of urease, ability to utilize citrate and malonate, and inability to hydrolyze gelatin or arginine (9).

Phenon 15 comprised three strains presumptively identified as *Erwinia* spp. but may, in fact, prove to be *Enterobacter agglomerans* (14), a taxon incorporated into the *Erwinia herbicola* group (8, 9). Phenon 16 shared characteristics of *Alcaligenes* (21), i.e., *Alcaligenes bronchiseptica* (36). Phenon 17, comprising five strains, was presumptively identified as *Pseudomonas* spp. from descriptions provided by Doudoroff and Palleroni (11). Phenon 18 could not be identified with the diagnostic keys and tables available in the literature. These bacteria were generally nonpigmented, nonmotile, gram-negative rods, catalase positive and capable of degrading starch, gelatin, and esculin and of growing at temperatures in the range 10 to 42°C. However, the strains exhibited variable oxidase reactions and a weak oxidative attack of glucose, indicating that the cluster may be *Acinetobacter* spp. (39).

Phenon 19, comprising two strains, was identified presumptively as *Chromobacterium lividum* on the basis of micromorphology, pigmen-

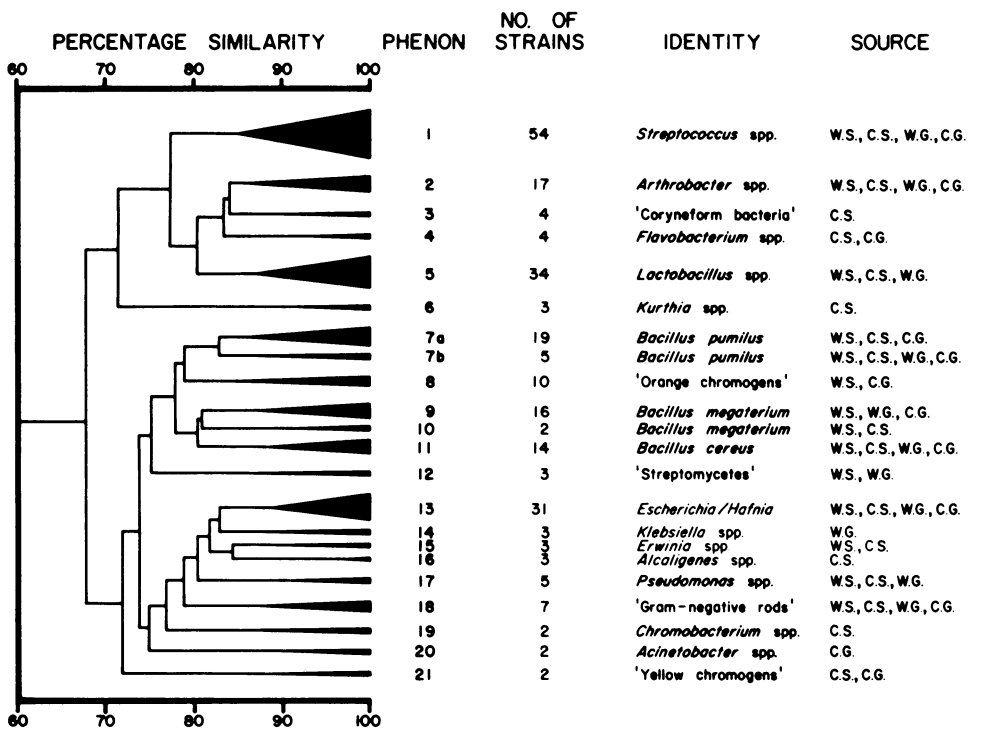

FIG. 1. *Dendrogram prepared from a numerical taxonomy analysis of data for strains isolated from waterfowl. The bacterial strains revealed similar clustering when both the simple matching (S_{SM}) and the Jaccard (S_J) coefficients were used. Results obtained with the S_J coefficient are shown. Groups were identified to the species level where possible. The original source of the strain is also listed (W.S., wild swan; C.S., captive swan; W.G., wild goose; C.G., captive goose; see text).*

tation, and biochemical properties, corresponding to the description of Sneath (47). The organisms were grouped with *C. lividum* rather than with *C. violaceum* primarily on the basis of esculin hydrolysis and utilization of various carbon sources. However, both strains differed from *C. lividum* in that the upper growth range was 42°C, more characteristic of *C. violaceum*. Phenon 20, with two strains, was tentatively identified as *Acinetobacter,* from the descriptions of Reyn (39). With the exception of ability to grow at 42°C, these characteristics coincided with the description of *A. calcoaceticus* (30). Phenon 21 represented an aggregate of yellow-pigmented, nonmotile, gram-positive or gram-variable rods, related to the coryneform group (41).

DISCUSSION

The aerobic, heterotrophic bacteria, i.e., those strains other than enterics, were predominantly *Streptococcus, Lactobacillus,* and *Bacillus* spp. Of the entire set of 356 strains, 132 strains (37%) proved to be enteric bacteria. The *Enterobacteriaceae* strains included *Escherichia, Enterobacter, Citrobacter, Klebsiella, Proteus, Hafnia,* and *Erwinia* spp. The enteric, *Streptococcus, Lactobacillus,* and *Bacillus* groups have been isolated by many investigators from the avian gut or feces, or from both (3, 16, 43–45).

Ninety-four of the *Enterobacteriaceae* strains were readily identified by using the API-20E diagnostic procedure; these represented nine species. *E. coli* proved to be the predominant species among the enterics found in the gut of the waterfowl, a situation similar to that of most warm-blooded animals, including humans. However, only five IMViC (indole, methyl red, Voges-Proskauer, citrate) typing patterns (1) considered to be representative of the *Enterobacteriaceae* were detected among the strains isolated from the waterfowl. In humans, 11 of the 16 possible IMViC patterns are usually recovered (16). Thus, as has been noted for many warm-blooded animals, swans and geese were found to carry a less diverse spectrum of species of *Enterobacteriaceae* than humans. Clearly, of the total aerobic and facultatively anaerobic flora, *E. coli* was not predominant, although of the enterics it was the major representative.

Salmonella and *Shigella* spp. were not encountered, even though extensive sampling and application of a variety of enrichment and selective media were used (24). It is concluded that healthy waterfowl in the wild, away from polluted environments, do not harbor these enteric pathogens. If they are present, they are certainly in numbers too small to be detected by methods used in this study.

The only aerobic cocci recovered and identified from the waterfowl were group D streptococci. Enterococci and lactobacilli are known to be associated with the gut contents of poultry (13, 43–45). *Bacillus* spp., i.e., phena 7a, 7b, 9, 10, and 11, a total of 56 strains, accounted for approximately 16% of the total set of strains recovered in large numbers from all of the swans and geese examined.

Representatives of other genera, such as *Flavobacterium* and *Arthrobacter* (see Table 2), were isolated in smaller numbers from most but not all, of the birds studied. Flavobacteria are commonly found in animals and have been reported to be among the earliest colonizers of many newborn animals (32, 42). *Arthrobacter*, however, is generally considered to be an inhabitant of soil and is not recognized as a colonizer of the alimentary tract. Nevertheless, strains of this genus were routinely isolated from aseptically autopsied colons of swans and geese, as well as from the feces of these animals. Such organisms might, indeed, be survivors from soil

TABLE 2. *Aerobic and facultatively anaerobic bacteria found in the gut of waterfowl*[a]

Genus and species	Phenon	Wild swans		Wild geese		Captive swans		Captive geese	
		No.	%[b]	No.	%	No.	%	No.	%
Streptococcus spp.	1	7	10	6	10	24	17	17	25
Arthrobacter spp.	2	2	3	1	2	7	5	7	10
Coryneform bacteria	3	0	0	0	0	3	2	0	0
Flavobacterium spp.	4	0	0	0	0	4	3	1	1
Lactobacillus spp.	5	9	13	15	25	11	8	0	0
Kurthia spp.	6	0	0	0	0	3	2	0	0
Bacillus pumilus	7a	9	13	2	3	3	2	7	10
Bacillus pumilus	7b	1	1	0	0	1	1	1	1
Bacillus megaterium	9	5	7	1	2	0	0	10	15
Bacillus megaterium	10	1	1	0	0	1	1	0	0
Bacillus cereus	11	5	7	1	2	6	4	2	3
Total *Bacillus* spp.		21	29	4	7	11	8	20	29
Streptomyces	12	2	3	1	2	0	0	0	0
Escherichia coli		11	16	9	15	45	31	1	1
Escherichia/Hafnia	13	6	9	5	8	16	11	3	4
Hafnia		0	0	2	3	0	0	1	1
Citrobacter freundii		0	0	0	0	1	1	2	3
Klebsiella pneumoniae	14	0	0	4	7	4	3	1	1
Klebsiella ozaenae		0	0	0	0	0	0	2	3
Klebsiella rhinoscleromatis		2	3	0	0	0	0	0	0
Proteus morganii		0	0	1	2	0	0	0	0
Enterobacter cloacae		0	0	4	7	0	0	2	3
Enterobacter agglomerans (*Erwinia* spp.)	15	2	3	0	0	6	4	2	3
Total enterics		21	30	25	42	72	50	14	21
Alcaligenes spp.	16	0	0	3	5	1	1	0	0
Aeromonas spp.		0	0	0	0	2	1	0	0
Pseudomonas spp.	17	2	3	1	2	3	2	0	0
"Gram-negative rods"	18	1	1	4	7	1	1	1	1
Chromobacterium spp.	19	0	0	0	0	2	1	0	0
Acinetobacter spp.	20	0	0	0	0	0	0	2	3
"Orange chromogens"	8	5	7	0	0	0	0	5	7
"Yellow chromogens"	21	0	0	0	0	1	1	1	1

[a] Includes all strains identified, both by API-20E and by numerical taxonomic analysis.
[b] Percentage of identified strains in each waterfowl type.

ingested along with food by the waterfowl.

The *Arthrobacter*, *Flavobacterium*, and other species, tentatively classified as coryneform bacteria, displayed a relatively high degree of phenotypic similarity, clustering at $S \geq 83\%$, based on results of analyses using the S_{SM} coefficient and average linkage for clustering. The taxonomic status of these genera is unclear, and their heterogeneous nature has been noted by others (4, 25, 27, 34). *Kurthia* spp., i.e., phenon 6, was not observed to be closely related to any other phenetic grouping, an observation also made by Jones (25).

A number of phena, notably, 3, 8, 18, and 21, could not be identified and classified unequivocably with genera and species described in the literature. Thus, it must be concluded that some of these phena represent as yet undescribed taxa. Two are groups of chromogenic bacteria. Phenon 8, the orange chromogens, when compared with pigmented bacteria recently isolated from Chesapeake Bay water and sediment and numerically classified by Austin and Colwell (manuscript in preparation), did cluster with an orange chromogen group subsequently identified as coryneforms. However, the other taxon of pigmented bacteria isolated from waterfowl, phenon 21, was not found to be closely allied with any other phenon associated with birds, either captive or in the wild. Neither could this taxon be equated with any of the yellow chromogens previously isolated by Austin et al. (2).

In conclusion, it is clear that swans and geese do possess a commensal flora of the gut, in terms of aerobic and facultatively anaerobic, heterotrophic bacteria. Five genera, *Streptococcus*, *Arthrobacter*, *Bacillus*, *Escherichia*, and *Enterobacter*, were positively identified as being present in the gut of both captive and wild swans and geese (see Table 2). Thirteen other genera were also recovered from the alimentary tract of wild or captive geese or swans (see Table 2). Dietary and environmental factors were not observed to alter significantly the spectrum of bacterial species present in intestinal tracts of swans and geese, even though such factors were associated with significant differences in the total numbers of these bacteria in the gut of waterfowl (24).

Among the facultatively anaerobic and fermentative groups of bacteria, the fecal coliforms (64 strains), i.e., *E. coli* type I, and fecal streptococci (54 strains) were the most numerous and proved to be the best indicators of fecal contamination. Since fecal coliforms and fecal streptococci are also considered to be the best indicators of animal and human fecal contamination, the impact of migratory waterfowl, namely, swans and geese, on aquatic ecosystems cannot be separated from that of human and domestic animals. Thus, isolation of fecal coliforms or fecal streptococci or both in large numbers cannot be considered to be indicative of human, domestic animal, or migratory waterfowl effects specifically. That is, it is not possible to "fingerprint" avian fecal pollution on the basis of the aerobic and facultatively anaerobic bacterial species found in given bodies of water. Furthermore, the "fecal streptococci/fecal coliform ratio," as proposed by Geldreich et al. (16) and discussed elsewhere (24), does not provide a reliable indication of avian fecal pollution. The best indication of public health hazard is the isolation and enumeration of specific human and animal pathogens. From results of studies accomplished to date, it is concluded that healthy, migratory waterfowl in the wild and not inhabiting polluted environments will not harbor significant numbers of *Salmonella* and *Shigella* spp. However, whether bacteria present in the healthy migratory waterfowl are pathogenic, as, for example, enteropathogenic *E. coli*, or potentially pathogenic, perhaps as opportunistic pathogens, such as *Klebsiella*, *Erwinia*, *Aeromonas*, and related species, remains to be determined. The obligately anaerobic bacterial flora of wild and captive swans and geese has not yet been fully characterized, although some information has been gathered (24). The anaerobes may provide the "indicator" species sought for avian fecal pollution, but the evidence to date is not encouraging.

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