Preliminary Studies of the Succession of Bacterial Genera Involved in the Maceration of Some Birds

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Maceration is the rotting away, in water, of tissues from vertebrate skeletons. Although this skeleton-recovery method is ancient in origin and has been satisfactorily employed for some time (2), little or no work has been done to determine the genera of bacteria involved in the decay process.

The present investigation is limited to the succession of bacterial genera present in the maceration of *Columba livia*, domestic pigeon (Columbiformes), and *Gallus gallus*, domestic chicken (Galliformes). Both species were chosen because of their convenient size and availability.

Although this is essentially a bacterial ecology study, the results may be of potential value to ornithologists in terms of understanding and facilitating the maceration process, and as a possible taxonomic tool, using characteristic patterns of bacterial succession as an aid in avian taxonomy.

Materials and Methods

The birds used in this study were killed by etherizing, then immediately prepared for maceration by being skinned, eviscerated, sexed and weighed. Each carcass was placed in a sterile glass Mason jar with lid. Enough sterile distilled water was added to cover the carcass. The birds were stored at a constant temperature of 37°C. throughout the study. The water in the jars was poured off and enough fresh sterile water to cover the carcass was added each week to prevent inhibition of bacterial growth from metabolic waste products. Each week before pouring off, 1 ml. samples of water from the macerating birds were aseptically obtained. Serial dilutions of 10^{-4}, 10^{-5}, 10^{-6} were used for plating. Tests necessary for identification were made based on physiological activities as described in a bacteriological key (1).

In order to determine the effects of single or specific groups of bacterial genera as opposed to the total flora, twenty-nine chickens were prepared for maceration, placed in jars of water and sterilized by autoclaving. Before using this procedure for large number of birds it had been determined that autoclaving did not affect the bacterial flora. Autoclaved birds which were inoculated with water from unautoclaved birds produced patterns of succession nearly identical to those of unautoclaved birds.

After autoclaving, twenty-one of the birds were divided into groups of threes. Each group was inoculated with one of the following seven genera: *Aerobacter*, *Bacillus*, *Clostridium*, *Escherichia*, *Flavobacterium*, *Proteus* and *Pseudomonas*. The selection of cultures was based on the number of appearances of these genera in the different birds studied, assuming their predominance to be an indication of their importance. The remaining eight birds were divided into: group 1, consisting of

three birds; group 2, consisting of three birds; and group 3, consisting of two birds. Group 1 was inoculated with the genera *Aerobacter, Clostridium, Escherichia* and *Pseudomonas.* Group 2 was inoculated with the genera *Bacillus, Clostridium, Escherichia, Flavobacterium, Proteus* and *Pseudomonas.* Group 3 was inoculated with all seven of the genera previously listed.

The bacteria used for the inoculations were obtained in the following manner: broth cultures of each genus were prepared using one loopful of culture per 10 ml. of nutrient broth. These cultures were incubated at 37°C. for twenty-four hours. Each broth culture was centrifuged for two ten-minute periods at high speed and resuspended twice in physiological saline solution. 1 ml. of the second saline suspension of each pure culture was used as the inoculum.

After inoculation the combined bird, jar, lid and water weight was recorded. The birds were stored at 37°C. in a closed incubator. Each week the jars of birds were weighed, the water poured off and a measured quantity of sterile distilled water added.

The effectiveness and importance of the bacterial genera in the maceration process was determined by the weight loss in grams of the birds each week over an eleven week period.

Results and Discussion

The succession pattern for four pigeons over a four week period (Table 1) showed the genera *Aerobacter* and *Alcaligines* were present the entire time. The genus *Clostridium* was present in the second through the fourth week and the genus *Escherichia* was present in the second through the fourth week with the exception of pigeons 7 and 9. The genera *Bacillus, Flavobacterium,* and *Streptococcus* appeared only intermittently.

TABLE 1

Succession Pattern of Bacterial Genera Present in Pigeons

Bacterial genera	week 1				week 2				week 3				week 4			
	1	7	8	9	1	7	8	9	1	7	8	9	1	7	8	9
Aerobacter	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Alcaligines	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Bacillus									x				x			
Clostridium					x	x	x	x	x	x	x	x	x	x	x	x
Escherichia		x		x	x	x	x	x	x	x	x	x	x	x	x	x
Flavobacterium													x			
Streptococcus									x				x			

The succession pattern for four chickens over a four week period (Table 2) showed the genus *Escherichia* was present in all birds for the entire time. The genus *Pseudomonas* was present the entire time for chickens A and C but only in the third and fourth weeks for chickens 1 and 4 and only one week for chickens A and C. With the exception of one appearance in the fourth week for chicken 1 the genus *Clostridium* appeared only in chickens A and C. The genera *Aerobacter, Alcaligines* and *Flavobacterium* appeared intermittently in all four chickens.

TABLE 2

Succession Pattern of Bacterial Genera Present in Pigeons

Bacterial genera	week 1				week 2				week 3				week 4			
	A	C	1	4	A	C	1	4	A	C	1	4	A	C	1	4
Aerobacter	x	x								x			x	x		
Alcaligines			x		x		x			x						
Bacillus			x	x	x	x					x	x			x	x
Clostridium					x	x			x	x			x	x	x	
Escherichia	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Flavobacterium							x						x			
Proteus			x	x			x	x							x	x
Pseudomonas	x	x			x	x				x	x	x	x	x	x	x

With the exception of the genus *Escherichia* the birds inoculated with a single culture failed to lose more than 4 grams between any weighing. In contrast to this the birds inoculated with groups of bacterial genera exhibited noticably larger weight losses, (Tables 3). Group 2 showed the largest amount of weight loss.

TABLE 3

Succession Pattern of Bacterial Genera Present in Chickens
Average Weight Loss in Grams of Chicken Specimens

Bacterial genera	times weighed					total wt. lost
	1	2	3	4	5	
Aerobacter	2	1	2	4	2	11
Bacillus	2	3	2	3	7	12
Clostridium	1	0	0	3	1	5
Escherichia	1	2	2	8	3	16
Flavobacterium	3	1	1	2	1	8
Proteus	1.5	2	1	3	5	12.5
Pseudomonas	2	1	3	4	5	13
*Group 1	2.5	2	4.5	6	1.5	16.5
*Group 2	5.7	24	4.3	13.3	7	54.3
*Group 3	3	2.5	2.5	8	7	23

*Group 1: Aerobacter, Clostridium, Escherichia, Pseudomonas
*Group 2: Bacillus, Clostridium, Escherichia, Flavobacterium, Proteus, Pseudonomas
*Group 3: Aerobacter, Bacillus, Clostridium, Escherichia, Flavobacterium, Proteus, Pseudomonas

In a comparison of the over-all succession patterns of pigeons and chickens the greatest differences were the appearance of the genus *Pseudomonas* in only chickens and the appearance of the genus *Streptococcus* in only one pigeon. There were also some differences in the genera present throughout the maceration and those appearing only part of the time. For all the pigeons the genera *Aerobacter* and *Alcaligines* were present the entire four weeks as compared to their intermittent appearance in only chickens A and C. The genus *Bacillus* was more preva-

lent in chickens than in pigeons. The genus *Clostridium* was present in the second through the fourth week for all pigeons but for only chickens A and C.

The greatest similarities between the succession patterns for the two species of birds were the infrequent appearances of the genus *Flavobacterium* and the repeated appearances of the genus *Escherichia.*

Age differences may have been a factor which contributed to these variations in succession patterns. The pigeons were all adults and the chickens although comparable in size were approximately five weeks old.

The results of inoculating sterile birds with pure cultures indicated that single genera are less effective than groups of genera. It was assumed that the group containing all the cultures would show the largest weight loss, however, the fact that it showed less weight loss than group 2 might suggest an antagonism between certain genera within the group.

The genus *Aerobacter* was present in the groups which lost the least weight and was not present in the group with the largest amount of weight loss. Possibly this genus inhibited the growth of other genera within groups 1 and 3.

The results of the individual inoculations and the survey of bacterial genera present over a given period of time suggest that groups of different bacterial genera with definite patterns of succession rather than a single genus are responsible for the maceration process. The genera *Aerobacter, Alcaligines, Clostridium, Escherichia* and *Pseudomonas* appear to be the most important in the maceration of the birds observed in this study. (Fig. 1).

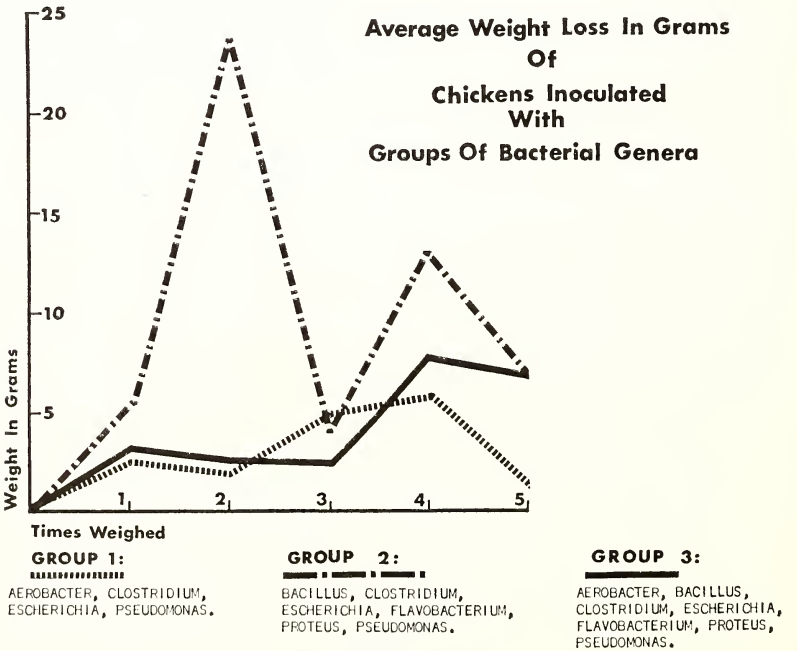

Figure 1.

Presently there is a paucity of studies in bacterial ecology because most of the current work in Bacteriology is on the molecular level. While this is extremely important it is also necessary to investigate the interaction of the organism with its natural environment in order to more fully understand its activities on the molecular level.

Work of this type is of immediate value to persons desiring bird skeletons for study. There is also a possibility that succession patterns may be specific enough for different orders of birds to use them as a taxonomic tool for classification.

Literature Cited

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2. Hawon, J. H., 1964. The technique of preparing bird skeletons for study by maceration. Am. Biol. Teacher, 26 (6) : 428-431.