UNUSUAL BACTERIAL FLORA IN LEACH'S STORM-PETREL, OCEANODROMA LEUCORHOA.

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The origins and composition of bacterial flora in seabirds is a relatively underexplored area. Pelagic seabird species such as Leach's storm petrel have access to an inoculum of bacteria limited to its prey and the water column. There is an extremely high bile titre in the stomachs and intestines of these birds (Place et al., MDIBL Bulletin, 1986), and we expect that this imposes severe selective pressures on the bacterial population that ultimately colonizes their intestines. In a separate study (Place, MDIBL Bulletin, 1990), we have found that Leach's Storm Petrel chicks can assimilate approximately 35% of the chitin that is prominent in their diets. Since many marine bacteria are able to initiate biodegradation of chitin, we also considered that the bacterial flora could contribute to the process of chitin degradation in this species.

A report by Ogg et al. (Applied and Environmental Microbiology, 55: 95-99,1989) has established that 20 species of waterfowl harbor and disseminate toxigenic <u>Vibrio cholerae</u> as a minor but potentially very important component of their hindgut flora. In addition, Lee et al. (J. Appl. Bacteriol. 52: 281-291, 1982) found <u>V. cholerae</u> in 6% of gulls (<u>Larus</u> spp).

Our goals were to establish the identity of the major bacterial components in juvenile and adult Leach's storm petrels, and to determine whether these bacteria contribute to the digestion of chitin in this species. Methods for enrichment of \underline{V} . Cholerae were employed in order to confirm or eliminate the possibility that Leach's storm petrel harbors this pathogen.

Specimens (fecal, proventricular or burrow samples) were collected aseptically and transferred to preweighed sterile microcentrifuge tubes. A 10% (w/v) suspension of material was homogenized by rotating a tight fitting pestle in the tubes. The supernatant was diluted appropriately and plated on to sterile selective and diagnostic media.

Difco culture swabs (#9343-26-7) were used to access cloacal feces in live birds, which were then released. The swabs were transported to the laboratory or island work area and used to inoculate media. Samples were placed in tubes containing 5 ml of alkaline peptone water for enrichment of V. cholerae. The air/medium interface was sampled after overnight incubation.

The following differential plating media were used:

- 1. MacConkey lactose
- 2. Trypticase Taurocholate Gelatin (TTG)
- 3. Gelatin agar
- 4. Trypticase Citrate Bile Sucrose (TCBS)
- 5. Salmonella/Shigella agar
- 6. Tryticase Soy Agar, with 1% sheep blood
- 7. CDC Anaerobic Blood.

Samples were spread or streaked on media, and incubated at $37~^{\rm O}{\rm C}$ either aerobically or in Gaspak Systems (BBL) which were equilibrated with anaerobic or microaerophilic atmospheres by means of Gaspak plus or Campypak plus systems respectively.

A total of 76 juvenile birds were sampled using the cloacal swab technique. Wing chord measurements and weights of these birds were recorded. Eight adult birds, which were captured during their sorties to feed young birds, were sampled and measured as above. Three birds were sacrificed by carbon dioxide asphyxiation, the gastrointestinal tracts were removed intact and the contents were sampled quantitatively. In addition, samples were collected from the interior surfaces of three representative nesting burrows.

To date, preliminary characterization of the flora from these birds has been completed. In summary, very few nestlings and none of the adult birds contained lactose positive (potentially coliform) or sulphide positive (<u>Salmonella</u>) isolates although these were prevalent in the burrow wall samples.

The majority of the birds (53/84, 63%) contained isolates that scored sucrose positive and were positive for bile selection. In birds where these bacteria occurred, the bacteria constituted the majority of the viable microbes observed. Surprisingly, these isolates were found to be Gram positive facultative anaerobic bacteria (probably Streptococcus or Lactobacillus), with extreme resistance to bile. No Vibrio cholerae isolates were found.

It is highly unusual that the majority of avian gut flora are Gram positive. Currently, biochemical testing is underway to determine the identity of this unusual bacterial flora component, and future studies will determine the origin and mechanisms of maintenance of these bacteria in the storm-petrel population, as well as their capacity to digest chitin.

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