Further Investigation into the Biology of *Macrorhabdus ornithogaster*

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Introduction

*Macrorhabdus ornithogaster* is an anamorphic ascomycetous yeast. 

*M. ornithogaster* is capable of infecting many species of birds, including parrots, finches, and poultry. Most infections do not result in an outwardly detectable disease, however, infections in individuals of some species can cause illness and death. Recent infection trials in chickens show that infection with *M. ornithogaster* results in mucosal hypertrophy and a heterophilic followed by a lymphoplasmacytic inflammation of the colonized mucosa.

*M. ornithogaster* is only found in the narrow zone (the gastric isthmus) between the proventriculus and ventriculus in the live bird. It is a fastidious organism and *in vitro* growth requires cell culture medium (pH 3-4), containing serum and one of a few sugars and a microaerophilic environment.

There is still much to learn about this organism. The host range and pathogenicity of *M. ornithogaster*, particularly in captive finches and in wild birds in Australia, is incompletely known. There is also a report of an organism outwardly resembling *M. ornithogaster* in the nasal secretions of a cat with an upper respiratory infection and in a bronchoalveolar lavage of a dog. However, the letter containing this report did not describe how the author concluded that the organism that he observed was in fact *M. ornithogaster*. Whether this organism was *M. ornithogaster* and whether it can grow in mammals remains to be proven.

*M. ornithogaster* is difficult to treat. It has only been found to be susceptible to amphotericin B given by gavage at 100 mg/kg twice a day for 30 days. Nystatin stopped *M. ornithogaster* shedding in a canary, but had no impact on shedding in Australian budgerigars. Less expensive and more convenient methods are needed for the treatment of individual birds and large collections of birds.

*M. ornithogaster* was defined as a single species in its own genus, by phylogenetic comparison of its rDNA sequence to those of other yeasts. This raises the question as to whether all the organisms morphologically defined as *M. ornithogaster* are the same or if there are genetic variants of *M. ornithogaster* or possibly other *Macrorhabdus* species. Additional studies of *M. ornithogaster* from different species of birds from different continents are needed.
The purposes of this investigation were to determine the prevalence of *M. ornithogaster* infections in three species of wild and captive-raised finch, to compare rDNA sequences from *M. ornithogaster* isolates from multiple finch species, determine if low toxic chemicals were capable of inhibiting *M. ornithogaster* growth *in vitro* and determine if mice could be infected with *M. ornithogaster* by oral or intraperitoneal inoculation.

**Materials and Methods**

Captive-raised zebra finches (*Taeniopygus bichenovi*) and budgerigars (*Melopsittacus undulates*) and captive-raised and wild caught European goldfinches (*Carduelis carduelis*) and green finches (*Carduelis chloris*) were examined in this study. Birds either died spontaneously or where euthanized. Infection was determined by examining scrapings of the gastric isthmus microscopically.

*M. ornithogaster* was grown from semipurified organisms from the isthmus of budgerigars as previously described.² RDNA was amplified directly from semipurified organisms or from cultured organisms, sequenced and the sequences compared as previously described.¹ The growth of a budgerigar isolate in multiple dilutions of amphotericin B, potassium sorbate, sodium benzoate, potassium benzoate, sodium acetate, fluconazole and nystatin was monitored over 5 days.

Three treatment groups of specific-pathogen-free mice where gavaged with one of two doses of *M. ornithogaster* or were given an intraperitoneal inoculation. Mice were monitored for 5 days after inoculation, euthanized and examined histologically for evidence of infection.

**Results**

1. A 100% infection prevalence was found in the zebra finches, green finches, and European goldfinches, no mater what their source.
2. Two budgerigars each from 6 show budgerigar breeders were examined for presence of *M. ornithogaster* infection. Five of six sources had 1 or two positive birds. Heavy infections were found in four birds from 2 collections and two of the birds from the one collection had an ulcerative gastritis.
3. Opportunistic sampling of four juvenile galahs with beak and feather disease presented to the Wildlife Health and Conservation Centre showed that they all were infected with an organism with the physical characteristics of *M. ornithogaster*. These birds were emaciated and had dilation of their proventriculus.
4. Sequences of 18S rDNA from *M. ornithogaster* from green finches and gold finches were identical but contained three unique point mutations as compared to the original *M. ornithogaster* sequence.
5. The majority of the 18S sequence of a zebra finch isolate varied markedly from the original *M. ornithogaster* sequence but was still clearly that of an anamorphic yeast.
6. A canary isolate shared blocks of sequence identical to that found in the green and European goldfinches and other blocks of sequence identical to that found in the zebra finch.
7. Amphotericin B, potassium sorbate, sodium benzoate, potassium benzoate, sodium acetate, and nystatin, but not fluconazole, inhibited *M. ornithogaster* growth at concentrations that might be achieved *in vivo*.

8. *M. ornithogaster* was not found in the mice five days after inoculation.

**Conclusions**

Based on these findings it appears that *M. ornithogaster* infection is widespread in some and perhaps many wild and captive-raised Australian finches. As some finch populations are declining in the wild, examination of wild native Australian finches for infection is indicated. Wild galahs from the Sydney area are also infected with *M. ornithogaster* or a similar organism.

Minor and massive 18S rDNA sequence differences were found in Australian isolates as compared to the originally reported 18S rDNA sequence of *M. ornithogaster*. The massive DNA sequence differences appear to be the result of a two step recombination event, as opposed to an accumulation of point mutations over time. The mechanism for such an event is under investigation. These differences in sequence complicate the molecular characterization of this organism and its detection by molecular means.

The *in vitro* inhibition trials confirm previous reports of the susceptibility of *M. ornithogaster* to amphotericin and suggest that some strains may be susceptible to nystatin, while resistance may occur in others. The ability of low toxic chemicals to inhibit *M. ornithogaster* growth points to the possibility that other less expensive and more convenient treatment methods could be developed.

*M. ornithogaster* inoculation did not result in mouse infection. Based on this result and its fastidious growth requirements, it is unlikely that *M. ornithogaster* can infect simple stomached mammals.

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**References**


