

Phylogenetic analysis identifies the 'megabacterium' of birds as a novel anamorphic ascomycetous yeast, *Macrorhabdus ornithogaster* gen. nov., sp. nov.

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An organism commonly referred to as 'megabacterium' colonizes the gastric isthmus of many species of birds. It is weakly Gram-positive and periodic acid–Schiff-positive and stains with silver stains. Previous studies have shown that it has a nucleus and a cell wall similar to those seen in fungi. Calcofluor white M2R staining suggests that the cell wall contains chitin, a eukaryote-specific substance, and rRNA *in situ* hybridization demonstrates that it is a eukaryote. To characterize this organism phylogenetically, DNA was extracted from purified cells. rDNA was readily amplified by PCR with pan-fungal DNA primer sets and primer sets derived from the newly determined sequence, but not with bacteria-specific primer sets. Specific primer sets amplified rDNA from isthmus scrapings from an infected bird, but not from a non-infected bird or other control DNA. The sequence was confirmed to derive from the purified organism by *in situ* rRNA hybridization using a specific probe. Phylogenetic analysis of sequences of the 18S rDNA and domain D1/D2 of 26S rDNA showed the organism to be a previously undescribed anamorphic ascomycetous yeast representing a new genus. The name *Macrorhabdus ornithogaster* gen. nov., sp. nov. is proposed for this organism. The type material is CBS 9251^T (=NRRL Y-27487^T).

An organism commonly referred to as 'megabacterium' infects domestic birds (Huchzermeyer *et al.*, 1993; Mutlu *et al.*, 1997; Wieliczko & Kuczkowski, 2000; Schulze & Heidrich, 2001) as well as wild (Filippich *et al.*, 1993; Pennycott *et al.*, 1998) and companion birds (Dorrestein *et al.*, 1980; Baker, 1992; Gerlach, 2001). These long, slender organisms (2–3 × 20–80 µm) stain with silver stains and periodic acid–Schiff (PAS) and are weakly Gram-positive. The 'megabacterium' is found in the isthmus between the glandular and grinding stomach, where it grows on the luminal surface and may penetrate koilin (Dorrestein *et al.*, 1980; van Herck *et al.*, 1984). It is associated with a lymphoplasmacytic gastritis in poultry (Mutlu *et al.*, 1997)

and a chronic fatal wasting disease in companion birds (Gerlach, 2001).

The organism was originally thought to be a yeast because of its staining characteristics (Dorrestein *et al.*, 1980). Subsequently, van Herck *et al.* (1984) concluded that it was a bacterium, as they were unable to demonstrate cytoplasmic organelles or a nucleus. They did, however, show nucleus-like structures in Geimsa-stained organisms, but interpreted them to be 'granules'. Scanlan & Graham (1990) reported isolating a bacterium from the stomach of a budgerigar using standard microbiological techniques. The isolated bacterium, however, was smaller than the organism observed *in vivo* and was not characterized by PAS or silver stains. Attempts by other investigators to grow this organism with standard microbiological isolation techniques have been unsuccessful. However, Gerlach (2001) reported isolation of this organism on MRS medium, but was unable to maintain it past a few passages. Huchzermeyer *et al.* (1993) also reported isolating an organism from the proventriculus of ostriches using MRS agar. This organism had the same biochemical properties as the one isolated by

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Abbreviations: D1/D2, domains 1 and 2; PAS, periodic acid–Schiff; PNA, peptide nucleic acid.

The GenBank/EMBL/DBJ accession number for the partial 18S, ITS1, 5-8S, ITS2 and partial 26S rDNA sequence of *Macrorhabdus ornithogaster* is AF350243.

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