

Potentially pathogenic yeast isolated from the throat and cloaca of an Arctic colonial seabird: the little auk (*Alle alle*)

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Abstract Yeasts are a distinctive group of microfungi, but compared to other microorganisms, their ecological function and biodiversity are poorly known. This is especially so where polar ecosystems are concerned. With climate changes and increasing pollution levels in the Arctic, it can be anticipated that there will be an increase in the prevalence and diversity of fungi colonizing live organisms. With these changes, it is crucial to investigate and monitor species diversity and prevalence of fungi in this fragile environment. In this study, yeasts were examined from throat and cloaca of a small colonial seabird, the little auk (*Alle alle*), a keystone species in the Arctic ecosystem. Samples were collected from 94 adults and 17 nestlings in breeding colony in Magdalenefjorden (NW Spitsbergen) in 2009. In total, twelve species of yeast from eight genera were found in 12 % of the samples, with the *Dipodascus* genus being the most prevalent. All yeast species were found in the adults, but only one species, *Cryptococcus macerans*, was found in a single nestling. In individuals where fungus was isolated, it was only isolated from either the throat or the cloaca, except for two cases, where fungus was found in both throat and cloaca. The presence of yeast was not related to sex but age of the birds, with adults being more prone to colonization by yeasts than the nestlings. The relatively low prevalence and diversity of yeast in little auks suggest that these birds are random carriers of fungi, with minor health impacts.

Keywords *Alle alle* · Fungi · Little auk · Mycobiota · Spitsbergen · Yeast

Introduction

Microorganisms are ubiquitous throughout the biosphere and play a fundamental role in ecosystems. They promote nutrient cycling and mediate important interactions between the biotic and abiotic components of the environment (Paul 2007; Peay et al. 2008). Yeasts are a distinctive group of microfungi, growing on organic substrates of many different origins. They are cosmopolitan, occurring in various terrestrial and aquatic habitats, including such extreme environments as deep-sea hydrothermal vents, acidic continental waters, and polar soils (Gadanhó and Sampaio 2005; Gadanhó et al. 2006; Connell et al. 2008; Kutty and Philip 2008). The ecological function and biodiversity of yeasts are poorly known.

Some yeasts are considered to be normal biota in the gastrointestinal tract, skin, and urinary system of animals and humans (Barnett et al. 2000). However, others can be pathogenic, causing life-threatening infections when the host's biological homeostasis and resistance are disrupted (Ghannoum and Abu-Elteen 1990; Dynowska and Kisicka 2005a). Moreover, certain forms of yeasts that are neutral to some animal species may be pathogenic toward others, including humans (Dynowska and Kisicka 2005a). Thus, animals colonized by some neutral yeasts may be natural reservoirs of potential pathogens and moving between different habitats, and they can become vectors of the pathogens transmission (Dynowska and Kisicka 2005b; Tsiodras et al. 2008).

Given their anatomy and physiology, birds should be excellent hosts and/or vectors of fungi. The fungi can

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develop within the warm and humid respiratory tract and the gastrointestinal system. Spread of fungi into the bird's body may be facilitated by the birds' behavior. The fungi may enter the body through the throat, while the bird forages or performs self-preening and/or through the cloaca during copulations (Ledwoń and Szeleszczuk 2008). However, it appears that entry of fungi into the body does not induce any antimycotic activity (Dynowska and Dynowski 1996), and incidences of mycoses are sporadically reported (Redig 2003). Indeed, birds have been usually reported as fungal carriers (Dupont et al. 1994; Tsiodras et al. 2008). These reports, however, have focused on commercially bred birds (mainly poultry and ornamental birds from zoological and private gardens). Only few studies have focused on fungi in wild species.

The aim of this study was to evaluate species composition and prevalence of yeasts in a small seabird, the little auk (*Alle alle*). It is a zooplanktivorous species, breeding colonially in rock crevices in Atlantic coastal areas of the high Arctic (Stempniewicz 2001). Foraging at sea and breeding on land, little auks transport organic matter from the nutrient-rich sea to the nutrient-poor land. Due to their feeding ecology and the size of global population (the most numerous seabird in the North Atlantic with population estimated at 37 million breeding pairs), the little auk is considered a keystone species and important in sustaining the Arctic terrestrial ecosystem (Stempniewicz 2006; Stempniewicz et al. 2007). Knowledge of the prevalence of yeasts in polar areas is sparse, and this is particularly so for avian assemblages. With ongoing climate changes and increasing pollution levels in the Arctic regions (IPCC 2007), an increase in the prevalence and diversity of potentially pathogenic fungi colonizing live organisms is expected (e.g., Dynowska and Kisicka 2005a). It is therefore crucial to investigate and monitor the species diversity and prevalence of fungi in this fragile environment. Moreover, a general understanding of the prevalence of yeasts in avian hosts may have broad implications for various disciplines, including mycology and ornithology.

Materials and methods

Fieldwork was conducted in the breeding colony of the little auk at Magdalenefjorden, NW Spitsbergen (79°30'N, 11°00'E). This is one of the largest breeding aggregations of the species in the Svalbard Archipelago (Isaksen 1995). Throat and cloaca swabs (hereafter called samples) were collected from 94 breeding adults (48 males and 46 females, including 37 breeding pairs) and 17 nestlings of the sampled parents during the early chick rearing period in 2009. Nineteen adults were double-sampled (from both throat and cloaca) 12–14 days after the first sampling

because the original samples froze. Both frozen and repeated samples were analyzed except for the generalized linear model analyses, where the frozen samples were excluded. A sterile cotton swab (2.5 × 120.0 mm; HAG-MED, Rawa Mazowiecka, Poland) was inserted for 5 s into the throat. A second swab was inserted into the cloaca for 5 s as well. Each swab was placed in a tube containing 6 ml liquid agar and an antibiotic [gentamycin and chloramphenicol (0.1 %)] to prevent bacterial growth. The samples were stored at 4–10 °C until cultivation. This temperature range was chosen because the temperatures were high enough to keep the fungi alive but sufficiently low to inhibit further microbiological growth. Birds were individually marked with a metal ring (Stavanger, Norway). Blood sample (ca 10 µl) was taken from the brachial vein for molecular sexing. The blood samples were stored in 96 % ethanol until analysis. All the birds were released without harm after a few minutes of handling.

Assuming a wide taxonomic spectrum of fungi, various media were used to set up the macrocultures (liquid and solid Sabouraud's, Czapek-Dox's and glucose-potato media; BTL, Łódź, Poland). The initial macrocultures were run on liquid media with gentamycin and chloramphenicol (0.1 %) at room temperature (23–25 °C) for 7 days (to acclimate the samples from cool storage) then transferred to 37–40 °C (required for the growth of mesophilic fungi) for 2–3 weeks (Clayton and Midgley 1989; Dynowska and Kisicka 2005a; Dynowska et al. 2005). Once proliferated, the fungi were passaged onto solid media [Nickerson's medium modified according to Dynowska and Kisicka 2005a, b; with broth bouillon and serum in a proportion of 1:1] to visualize diagnostic traits (morphology, arrangement of the colony, and colors). Additionally, for diagnostic purposes, biochemical tests using standard strips (API 20C, API 20AUX, bioMérieux, France) were performed to analyze the assimilation and fermentation capacity of particular fungi (Freydiere et al. 2001). The following literature was used to identify the fungi species: Kreger-van Rij (1984), De Hoog et al. (2000), Kurtzman and Fell (2000), Howard (2003) and Kurtzman et al. (2011). Of the fungi cultivated, only yeast and yeast-like fungi were considered in the present study. Molds and dermatophytes were reported elsewhere (Wojczulanis-Jakubas et al. 2011; Dynowska et al. in prep.)

Molecular sexing was performed on DNA extracted from the blood using the Blood Mini kit (A&A Biotechnology, Gdynia, Poland), following evaporation of the alcohol. The CHD region was amplified with the primer pair 2550F and 2718R (Fridolfsson and Ellegren 1999) according to the PCR protocol described by Griffiths et al. (1998), at a 50 °C annealing temperature. The difference in the PCR product size (ca 200 bp) was clearly visible when separated on 2 % agarose gel.

A generalized linear model with binomial distributions and a probit function were used to test the effects of sex and age (independent factors) on the probability of yeast infection (binary-coded: “1”—presence, if at least one yeast species was present in the sample, and “0”—absence). Separate analyses were performed for each swab type to alleviate the problem of pseudoreplication caused by the repeated sampling of the same individuals (two types of swabs from the same individual). The significance of the relationships in the models was determined using the maximum likelihood test. The analyses were performed in STATISTICA 9.0 (StatSoft, Poland).

Results

Yeasts were recorded in 31 (12 %) of all 260 samples. A total of twelve species of yeast from eight genera were found (Table 1). The most frequently recorded genus was *Dipodascus* with four species, of which *D. albidus* occurred in 10 samples. *Rhodotorula glutinis* and *Cryptococcus macerans* followed, occurring in seven and three samples, respectively. *D. armillariae* and *D. ingens* were each found in two samples, and the remaining seven species (*Candida albicans*, *D. capitatus*, *Dipodascopsis tothii*, *Geotrichum candidum*, *Exophiala spinifera*, *Cr. laurentii* and *Saccharomyces bayanus*) were recorded once only. One species was found per sample, except for a single sample containing two species (*Dipodascus albidus* and *D. armillariae*).

All the yeast species were found in adults. Only one species (*Cr. macerans*) was found in a single nestling

Table 1 A checklist of yeast species recorded in throat and cloaca of the little auks in Magdaleneffjorden (NW Spitsbergen) with the number of individuals found to be colonized by fungi species

Species	Swab type	
	Throat (<i>N</i> = 130)	Cloaca (<i>N</i> = 130)
<i>Candida albicans</i>	1 A	–
<i>Cryptococcus laurentii</i>	1 A	–
<i>Cryptococcus macerans</i>	–	2 A + 1N
<i>Dipodascus albidus</i>	5 A	5 A*
<i>Dipodascus armillariae</i>	1 A	1 A
<i>Dipodascus capitatus</i>	1 A	–
<i>Dipodascus ingens</i>	1 A*	1 A*
<i>Dipodascopsis tothii</i>	1 A	–
<i>Geotrichum candidum</i>	–	1 A*
<i>Exophiala spinifera</i>	1 A	–
<i>Rhodotorula glutinis</i>	5 A*	2 A*
<i>Saccharomyces bayanus</i>	1 A*	–

Underlined species were recorded in birds for the first time ever
Asterisks indicate the samples that froze before the fungi cultivation.
A adult; N nestling

(Table 1). In the family with the yeast-positive nestling, the male parent was also yeast-positive in the cloacal sample but with different species (*D. ingens*). In the families with the yeast-negative nestling, the pattern of yeasts occurrence within the family members varied and was as follows: (1) both parents were positive but with different yeast species in the throat or cloaca (*N* = 2 families, *Rh. glutinis* in the throat vs *Saccharomyces bayanus* in the cloaca; *D. tothii* in the throat vs *D. albidus* in the cloaca), (2) only one parent was positive in the throat (*N* = 2; *Rh. glutinis* and *D. ingens*), (3) both parents were concordant with the nestling in lack of fungi both in throat and in cloaca (*N* = 12).

Four species were found in both swab types (*D. albidus*, *D. armillariae*, *D. ingens* and *Rhodotorula glutinis*), and other five species (*Candida albicans*, *Cr. laurentii*, *D. capitatus*, *D. tothii* and *Exophiala spinifera*) were found exclusively in the throat and the three remaining ones in the cloacal swabs (*Cr. macerans*, *Geotrichum candidum* and *Saccharomyces bayanus*; Table 1).

The fungi were recorded exclusively in the throat (*N* = 14) or cloaca (*N* = 12) in all the individuals examined. Only on two occasions, fungi were recorded from both the throat and the cloaca of the same individual, and in both cases, the species composition was different (*Cr. laurentii* vs *D. albidus* and *Cr. macerans* vs *Rh. glutinis*).

Testing the probability of yeast occurrence in the throat showed that adults were more likely to have been colonized than nestlings (15 vs. 0 % infected individuals respectively; LR test, $\chi^2 = 4.55$, *P* = 0.03), whereas the probability of yeast occurrence was similar in the two sexes (12 and 14 % adult males and females were infected; $\chi^2 > 0.001$, *P* = 0.99). For the cloaca swabs, no effect of sex or age was found on the presence of yeasts. The adults were just as likely to have been colonized as the nestlings (9 vs. 6 % infected individuals, respectively; LR test, $\chi^2 = 0.74$, *P* = 0.39); similarly, males were just as likely to have been infected as females (8 and 9 % adult males and females were infected; $\chi^2 = 0.67$, *P* = 0.41).

Of the 38 pairs of the frozen-repeated samples, the same negative result during both samplings was recorded in 29 pairs of the samples (with 16 from the throat, and 13 from the cloaca). For one pair of the samples (from the throat), the record of *Rh. glutinis* was repeated. In two other pairs, two species of yeasts (*Rh. glutinis* and *D. albidus* in the throat and cloaca, respectively) were found in the repeated samples, whereas the results were negative for the relevant frozen ones. In six other cases, the opposite pattern was observed. *D. ingens* was found in the frozen sample from the throat, and *D. ingens*, *D. albidus*, *G. candidum*, *Sacch. bayanus* and *Rh. glutinis* were present in the frozen samples from cloaca, but none of these species were found in the repeated ones.

Discussion

Prevalence of the yeast in the little auk was not high but noticeable. They were recorded in a quarter of the studied birds. *Dipodascus albidus* and *Rhodotorula glutinis* were the most frequently recorded of the twelve yeast species found. The former along with *D. armillariae*, *D. ingens* and *Exophiala spinifera* have never been reported from birds before, whereas *Rh. glutinis* has been reported from seabirds (Kutty and Philip 2008).

All the yeast species recorded are potentially pathogenic to humans. Four species have been classified to BioSafety level BS 1 (agents of minimal potential hazard to laboratory personnel and the environment) and three to BS 2 (agents of moderate potential hazard to personnel and the environment; De Hoog 1996). Three species that are known to cause serious health impact are particularly worthy of attention: (1) *Cr. macerans* causing cryptococcosis, one of the most serious, and usually fatal, diseases in birds (Rosario et al. 2005), (2) *Exophiala spinifera*, which can induce opportunistic infections of skin and subcutaneous tissues of humans and other mammals (Kettlewell et al. 1989; Harris et al. 2009), (3) *Candida albicans*, which can cause candidosis of the alimentary, respiratory and reproductive systems and skin of birds (Buck 1983; Dynowska and Dynowski 1996; Buck and Chabasse 1998).

While the isolation of fungi from the throat may be indicative of their accidental occurrence, records of fungi in the cloaca suggest that they have passed through or are still present in the bird's alimentary tract; hence, the bird may be a carrier or may be developing an infection (Casadevall and Pirofski 2000; Dynowska and Kisicka 2005a, b). However, the inconsistency between the throat and cloaca samples from the same individual indicates that patterns of infection are complex. Moreover, all the birds examined, including those with yeasts found in the cloaca, were in excellent body condition, and were observed to be behaving and breeding normally. Also, the chick positive for the occurrence of *Cr. macerans* in the cloaca survived to fledging. All this, together with not so high frequency of yeast records, suggests that the little auks examined could have been random carriers of the yeasts, with minor health impact.

The fungal composition of the frozen and repeated samples was consistent in 79 % of the pair-samples. However, fungi were recorded in six frozen samples but were absent in the repeated ones, and in two cases, the results were reversed. The discrepancy between pair-samples could have been due to limitations of the sampling method (relatively short time of swab insertion, 5 s). Some fungi could have been randomly collected or not collected when an individual was infected at a very low level. Moreover, some microfungi may not have been cultivated,

despite being viable, as is the case in some bacterial cultures (Ohtomo and Saito 2001). It is also worth emphasizing that some fungi can survive freezing, but as the freezing effect was unintentional and not controlled, it is difficult to discuss it conclusively. However, the apparent tolerance of the fungi to low temperatures might be an adaptation to polar conditions, and this clearly seems worth to be further explored.

Little auks may be colonized by yeasts in various ways, though the significant effect of age on the yeast record suggests that the birds need to spend a considerable time in the environment to become colonized. The fungi proliferating in marine and terrestrial habitats may passively penetrate the avian host through an orifice and/or mucous membrane (Dynowska and Kisicka 2005a, b). Colonization may take place both in the nesting crevice during incubation and chick rearing and in the water, while the bird is resting or foraging. Moreover, the latter can occur both during breeding in the high Arctic and during the wintering period in more southerly waters (e.g., in the Danish Straits). Given the non-concordance in the yeast species composition found within the families, their mutual transmission seems to be complex (see also Wojczulanis-Jakubas et al. 2011). It is possible, however, that the transmission would be more effective if the birds were indeed infected, with a direct impact on their health.

When indeed infected, little auks might further transmit the fungi into the environment via their excreta. Osono et al. (2002) showed that the diversity of the microfungal community is proportional to the nutrient content (phosphorus and nitrogen, in particular) in the area where the birds nest and is maintained for 2–3 years after a colony had been abandoned by the birds. The effect of the little auk mycobiota on the tundra is unknown, but it may be speculated that the fungi reported from little auks are likely to be present in the colony area.

Information on microfungi in Arctic birds is scarce (Del Frate and Caretta 1990; Muzaffar and Jones 2004; Butinar et al. 2007). This is mainly due to the ecological specificity of polar regions and their poorer microbiota compared to that of other climatic zones (Kutty and Philip 2008). Much more information is available from tropical, subtropical (Buck 1983; Buck and Chabasse 1998; Kutty and Philip 2008) and Mediterranean areas (Mancianti et al. 2002; Cafarchia et al. 2006) or from the boreal zone (Dynowska and Kisicka 2005a, b). The migration of potentially pathogenic fungi among habitats has been observed, but only recently, the importance of this biological regularity and its epidemiological significance in respect of wild birds has been emphasized (Tsiodras et al. 2008). The Arctic region is currently undergoing a dramatic climate change, with twofold higher increase in temperature compared to the global increase, and that trend is expected to continue

(IPCC 2007). As a consequence, reports of emerging and reemerging of many different organisms in the Arctic ecosystems are on the increase. The investigation into the microfungal community in little auks could therefore be one of the ways of monitoring the ongoing changes in the Arctic ecosystems.

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