J Vet Clin 28(2) : 254-257 (2011)

Journal of Veterinary Clinics
www.ksvc.or.kr

Isolation of *Pichia burtonii* from the Feces of an Enteritis Bearded Dragon (*Pogona vitticeps*)

Hyo-Min Kang, Jae-Ik Han, Sook-Jin Lee, Hye-Jin Jang and Ki-Jeong Na[1]

Veterinary Laboratory Medicine, College of Veterinary Medicine, Veterinary Medical Center, Chungbuk National University, Cheongju 361-763 Korea

(Accepted: April 04, 2011)

Abstract : A 2-year-old bearded dragon was referred to the Veterinary Medical Center at the College of Veterinary Medicine, Chungbuk National University with reduced activity and anorexia. On fecal examination, over growth of a bacteria and the proliferation of a yeast-like organism were found. The patient diagnosed with enteritis. By using fungal cultures and molecular typing, the yeast was identified as *Pichia (P.) burtonii*. The bearded dragon was treated with oral ketoconazole and trimethoprim/sulfamethoxazole. After 3 days, the dragon was recovered and fecal examination showed that the yeast had disappeared from the feces. The strain *P. burtonii* is supposed opportunistic pathogen in bearded dragon with enteritis according to its reports in a human. This report is the first paper about overgrowth of *P. burtonii* in a bearded dragon.

Key words **:** bearded dragon, enteritis, *Pichia burtonii*.

Introduction

Yeasts are considered as common commensals of reptiles, especially in the gastrointestinal tract (5). Typically, overgrowth of fecal microorganisms is a secondary, nonspecific finding associated with other underlying diseases, abnormal physiologic conditions, or administration of antimicrobial (12). However, microbial overgrowth may exacerbate underlying pathology (12) and mycotic agents that are harmless in healthy individuals may become opportunistic in immunocompromised patient (7). Yeast or fungal gastrointestinal infections are infrequent causes of anorexia in lizards (4).

Yeasts of Genus *Pichia* are widely distributed and found in natural habitats such as soil, fresh water, tree exudates, insects, plants and fruits and also as contaminants in a variety of foods and beverages. Some *Pichia* species contribute fermentation of food (11). Other species have been described as human pathogens (1, 8). This report describes a case of *Pichia (P.) burtonii* in the feces of a bearded dragon.

Case

History

A 2-year-old bearded dragon weighing 80 g was referred to the Veterinary Medical Center at the College of Veterinary Medicine, Chungbuk National University with reduced activity and anorexia (Fig 1). The clinical signs were first noticed 2 week prior to presentation and progressive slowly. No other abnormalities were detected on physical examination and the husbandry problem was ruled out by history taking.

Fecal examination and fungal culture

Wet-mount and direct smears of the feces were prepared for microscopic examination. The feces appeared grossly brownish. The smears contained many rod-shaped bacteria and yeast-like organisms (Fig 2). Because of the presence of many yeast-like organisms, the feces was cultured at 30°C for 10 days on sabouraud-dextrose agar supplemented with gentamicin (Becton Dickinson, Franklin Lakes, NJ 07417, USA). The plate showed the growth of white circular colony, which had smooth surface and irregular grooves at its margins, but rough surface

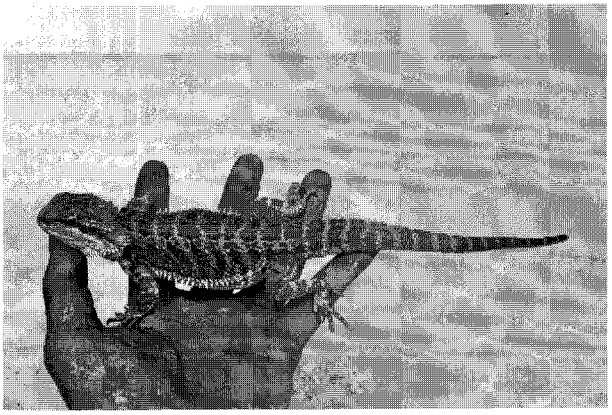

Fig 1. A 2-year-old bearded dragon with reduced activity and anorexia.

[1]Corresponding author.
E-mail : sigol@cbnu.ac.kr

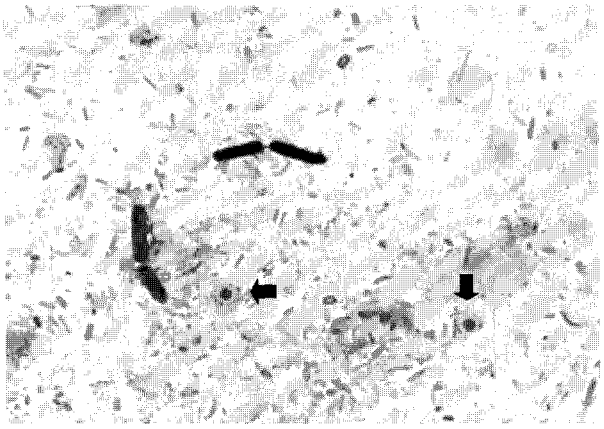

Fig 2. On fecal examination, proliferation of yeast-like organism (arrows) was detected (Diff-Quik stain, 1000×).

Fig 3. The result of subculture of feces on Sabouraud dextrose agar.

in the center (Fig 3). Microscopic examination of the colony showed hyphae and conidia that resemble those in the feces.

Fungal identification

The yeast was identified by molecular typing. The genomic DNA was extracted by vortexing with glass beads (10) and was amplified using the primers ITS1 and ITS4 as described previously (6). PCR amplification was performed in a total volume of 50 μL. The final reaction conditions were as follows: 50 mM KCl, 10 mM Tris-HCl (pH 8.3, 25°C), 1.5 mM $MgCl_2$, 200 μM of each dNTP, 100 ng of each primer, and 5 units of Taq polymerase (iNtRON Biotechnology, Sungnam, South Korea). The PCR was performed in a TaKaRa Thermal Cycler Dice (Takara Bio Inc., Otsu, Shiga, Japan) under the following conditions: an initial denaturation step at 94°C for 3 min, followed by 30 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 1 min, and a final incubation at 72°C for 3 min. The PCR product was separated by electrophoresis for 50 min at 100 V in a 2% agarose gel and was stained with ethidium bromide for visualization under ultraviolet light. The amplicon was sequenced using

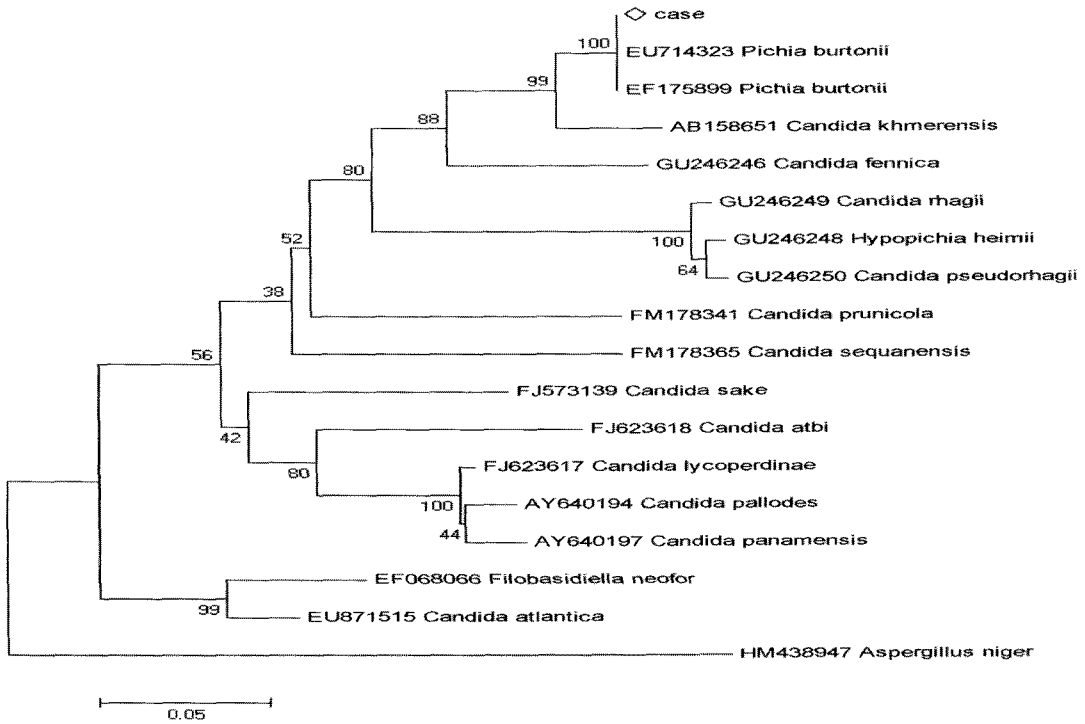

Fig 4. Neighbor joining phylogenetic tree based on the alignment of ITS region gene sequences of *Pichia burtonii* isolated from bearded dragon. *Aspergillus niger* was selected as an out-group. Sequence alignments were performed using ClustalX v.1.8, and MEGA4 v.4.02 was used for the phylogenetic analysis. Bootstrap percentage values are shown on the branches.

an ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit v3.1 (PE Applied Biosystems, Foster City, CA, USA). A comparison with the gene sequences deposited in GenBank revealed that the sequence of the isolated yeast was 100% similar to a *P. burtonii* sequence that had been deposited in Greece (GenBank accession number EU714323). Thus, we identified the yeast isolated from the bearded dragon as *P. burtonii*. Neighbor-joining phylogenetic analysis of the sequence also revealed that the yeast isolated from the bearded dragon was *P. burtonii* (Fig 4).

Treatment

The patient was treated with daily oral ketoconazole (30 mg/kg, Seoul, Korea) and trimethoprim/sulfamethoxazole (20 mg/kg, Seoul, Korea) for 3 days. By the fourth day, the clinical signs had disappeared, and yeast was no longer detected on fecal examination.

Discussion

This report describes a case of an anorexic bearded dragon with abnormal intestinal growth of *P. burtonii*. *P. burtonii* has previously been shown to be associated with fermentation (9). *P. burtonii* has also been shown to stimulate aflatoxin production of *Aspergillus flavus* in a dual culture (2) but little is known about its pathogenicity.

A study about yeasts in ninety-one reptiles described that yeasts were most often isolated from gastrointestinal tract and herbivore have yeasts more often than carnivore. In that study, however, no sufficiently reliable and practical criteria were established about association between positive culture results and disease (5). Although it is unclear whether overgrowth of *P. burtonii* contributed to the symptoms or was only secondary change, the dragon recovered after the medication with an antifungal agent, suggesting that this fungus might be linked to the hypodynamia of dragon.

In this study, a ribosomal DNA region spanning the ITS1 and ITS2 and the 5.8S gene was employed to identify and perform phylogenetic analysis, because this region has a low intraspecific polymorphism and a high interspecific variability (3).

References

1. Bakir M, Cerikcioğlu N, Tirtir A, Berrak S, Ozek E, Canpolat C. Pichia anomala fungaemia in immunocompromised children. Mycoses 2004; 47: 231-235.
2. Cuero RG, Smith JE, Lacey J. Stimulation by Hyphopichia burtonii and Bacillus amyloliquefaciens of aflatoxin production by Aspergillus flavus in irradiated maize and rice grains. Appl Environ Microbiol 1987; 53: 1142-1146.
3. Esteve-Zarzoso B, Belloch C, Uruburu F, Querol A. Identification of yeasts by RFLP analysis of the 5.8S rRNA gene and the two ribosomal internal transcribed spacers. Int J Syst Bacteriol 1999; 49: 329-337.
4. Funk RS. Anorexia. In: Reptile medicine and surgery. Philadelphia: Saunders. 1996: 346-348.
5. Kostka VM, Hoffmann L, Balks E, Eskens U, Wimmershof N. Review of the literature and investigations on the prevalence and consequences of yeasts in reptiles. Vet Rec 1997; 140: 282-287.
6. Makimura K, Mochizuki T, Hasegawa A, Uchida K, Saito H, Yamaguchi H. Phylogenetic classification of Trichophyton mentagrophytes complex strains based on DNA sequences of nuclear ribosomal internal transcribed spacer 1 regions. J Clin Microbiol 1998; 36: 2629-2633.
7. Nardoni S, Papini R, Marcucci M, Mancianti F. Survey on the fungal flora of the cloaca of healthy pet reptiles. Revue Med Vet 2008; 159: 159-165.
8. Otag F, Kuyucu N, Erturan Z, Sen S, Emekdas G, Sugita T. An outbreak of Pichia ohmeri infection in the paediatric intensive care unit: case reports and review of the literature. Mycoses 2005; 48: 265-269.
9. Takeuchi A, Shimizu-Ibuka A, Nishiyama Y, Mura K, Okada S, Tokue C, Arai S. Purification and characterization of an alpha-amylase of Pichia burtonii isolated from the traditional starter "murcha" in Nepal. Biosci Biotechnol Biochem 2006; 70: 3019-3024.
10. Van Burik JA, Schreckhise RW, White TC, Bowden RA, Myerson D. Comparison of six extraction techniques for isolation of DNA from filamentous fungi. Med Mycol 1998; 36: 299-303.
11. Villa-Carvajal M, Querol A, Belloch C. Identification of species in the genus Pichia by restriction of the internal transcribed spacers (ITS1 and ITS2) and the 5.8S ribosomal DNA gene. Antonie Van Leeuwenhoek 2006; 90: 171-181.
12. Wamsley HL. Dry-mount fecal cytology. In: Canine and feline cytology: A color atlas and interpretation guide. St. Louis: Saunders. 2010: 215-225.

장염을 나타낸 bearded dragon의 분변에서 *Pichia burtonii*의 분리

강효민 · 한재익 · 이숙진 · 장해진 · 나기정[1]

충북대학교 수의과대학 동물의료센터

요 약 : 2년령 bearded dragon이 활력 감소 및 식욕 부진으로 충북대학교 동물의료센터에 내원하였다. 분변 검사 결과 다수의 세균과 함께 효모의 증식이 관찰되어 장염으로 진단되었다. 진균 배양 및 분자생물학적 진균 동정 결과 증식된 효모는 *Pichia* (*P.*) *burtonii*로 확인되었다. 치료는 ketoconazole과 trimethoprim/sulfamethoxazole을 3일간 경구 투여하였다. 치료 3일 후 분변 내 효모는 사라졌으며, 이후 환자는 회복되었다. 본 증례와 사람에서의 보고를 종합하면 *P. burtonii* 는 장염이 있는 bearded dragon의 기회성 병원체로 추정된다. 또한 본 증례는 bearded dragon에서의 *P. burtonill*의 과증식에 대한 최초의 보고이다.

주요어 : Bearded dragon, 장염, *Pichia burtonii*