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First Report of Brain Abscess Associated with *Pseudozyma* species in a Patient with Astrocytoma

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A yeast-like strain was isolated from the brain abscess of a patient diagnosed with astrocytoma. Morphological and molecular analysis on D1/D2 domain in the 26S rRNA gene and internal transcript spacer region of the strain revealed that the strain belonged to the genus *Pseudozyma*. To the best of our knowledge, this is the first report on the isolation of a *Pseudozyma* strain from brain abscess. *(Korean J Lab Med 2010;30:284-8)*

Key Words : *Pseudozyma species, Molecular diagnostic techniques, Brain abscess*

INTRODUCTION

The *Pseudozyma* species are ustilaginomycetous yeasts and usually isolated from plants [1, 2]. Thus far, there are only 2 reports on the isolation of *Pseudozyma* strains from clinical specimens obtained from blood and central venous catheter [2, 3]. Recently, a yeast-like strain was isolated from a cerebral abscess sample, which was obtained from the operation room, of a patient diagnosed with astrocytoma. Morphologic and molecular analyses of the isolate revealed that it belonged to the genus *Pseudozyma*. Herein, we present the first report on the isolation of *Pseudozyma* strain from a cerebral abscess.

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CASE REPORT

A 78-yr-old male presented to a tertiary hospital with complaints of weakness in his right leg lasting for 2 months. Magnetic resonance imaging (MRI) revealed a 2 cm mass in the left frontal lobe. On hospital day 7, a stereotactic brain biopsy examination was performed, and pathological diagnosis suggested anaplastic astrocytoma. On hospital day 20, fever developed as high as 37.9℃. Blood analysis showed elevated white blood cell counts of 26,390/μL with 93.6% neutrophils and elevated C-reactive protein level of 23.8 mg/dL. We obtained 3 pairs of blood samples for microbial examination. After 3 days of incubation with BacT/Alert 3D system (bioMéieux, Durham, NC, USA), methicillin-resistant *Staphylococcus aureus* (MRSA) were detected in both of aerobic and anaerobic cultures. Intravenous (IV) vancomycin treatment was started. On hospital day 21, i.e., 14 days after brain biopsy, MRI study revealed a cerebral abscess at the biopsy site. On hospital day 22, the abscess was surgically removed in the operation room and the pus was

transferred to the clinical microbiology laboratory for culture study.

The sample was inoculated on blood agar, MacConkey agar, Sabouraud dextrose agar, thioglycollate broth, and phenylethyl alcohol blood agar. Many white medium-sized colonies were grown on blood agar but no colony developed on the MacConkey agar after 24 hr incubation for under 5% CO_2 at 35℃. The isolate was identified as MRSA. In addition, about 2-2.5 mm diameter, butyrous, soft, smooth to irregularly furrowed, creamy-white colonies with fringed margin by short mycelium were observed on Sabouraud dextrose agar after incubation for 3 days at 30℃ (Fig. 1A). Gram staining revealed a 1-1.5 μm×9-10 μm fusiform or cylindrical yeast with budding on short stalks (Fig. 1B). Elongated spindle-shaped fusiform blastoconidia were observed in the slide culture performed on Sabouraud dextrose agar (Fig. 1C). However, the species could not be identified by VITEK Yeast Biochemical Card (YBC; bioMérieux, Mrcy l'Etoile, France).

For molecular identification, DNA was extracted and purified by using DNA purification kit, DNeasy® Blood and Tissue Kit (Qiagen GmbH, Hilden, Germany). DNA was amplified with ITS4-Fungus PCR Forward (5′-TCC GTA GGT GAA CCT GCG G-3′) and ITS4-Fungus PCR Reverse (5′-TCC TCC GCT TAT TGA TAT GC-3′) primers for internally transcribed spacer (ITS) region [4], and Pseudozyma NL-1 (5′-GCA TAT CAA TAA GCG GAG GAA AAG-3′) and Pseudozyma NL-4 (5′-GGT CCG TGT TTC AAG ACG G-3′) primers were used for amplifying the D1/D2 domain of the 26S rRNA gene [5]. Direct sequencing of PCR products was performed using the ABI PRISM 3730xl Analyzer (Perkin-Elmer Applied Biosystems, Foster City, CA, USA). The obtained sequences were compared to the known sequences in the EMBL database (http://www.ebi.ac.uk/Tools/fasta33/). The highest sequence identity of 98.6% (1,299/1,317 bp) was obtained with the strains of *Pseudozyma* species (DMST 17136; GenBank accession number AB117962). The next highest identity of 91.2% was obtained with the *Ustilago altilis* strains (GenBank accession number AY740166).

Phylogenetic trees were constructed using Clustal X software, and the species deduced from the ITS and D1/D2 region sequence data was confirmed [6]. Phylogenetic analysis was conducted by using the neighbor-joining method and bootstrap analysis was performed [7, 8]. The TreeView program (http://taxonomy.zoology.gla.ac.uk/rod/treeview.html) was used to view the phylogenetic trees. Strain YCM09/6/F44 exhibited the highest sequence similarity with *Pseudozyma* sequences (Fig. 2).

Subsequent culture study of cerebral abscess was not performed because of the invasive nature of the sampling procedure in this case. The follow-up blood culture examinations did not reveal any conspecific yeast strain. On the hospital day 28, the patient became drowsy, and he developed ventilator-associated pneumonia with pulmonary consolidation due to MRSA. Radiation therapy was initiated due to rapid tumor growth. Patient's kidney function gradually worsened with increasing blood creatinine levels and his pulmonary function continued to deteriorate. These poor general conditions hindered the

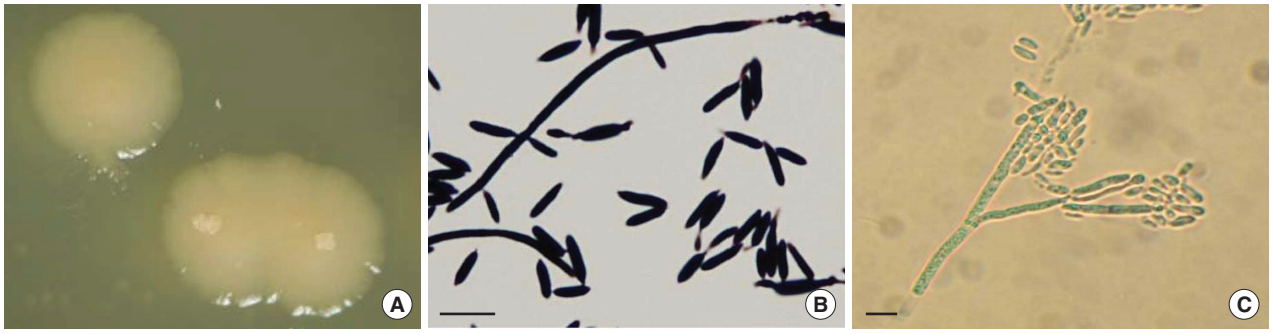

Fig. 1. (A) Colony morphology after 3 days of incubation on Sabouraud's dextrose agar at 30℃ and (B) gram staining showing fusiform yeast with budding (Gram stain, × 1,000). (C) Slide culture on Sabouroud's dextrose agar after 3 days of incubation at 30℃ in ambient air showing elongated spindle-shaped blastoconidia. Scale bar=10 μm.

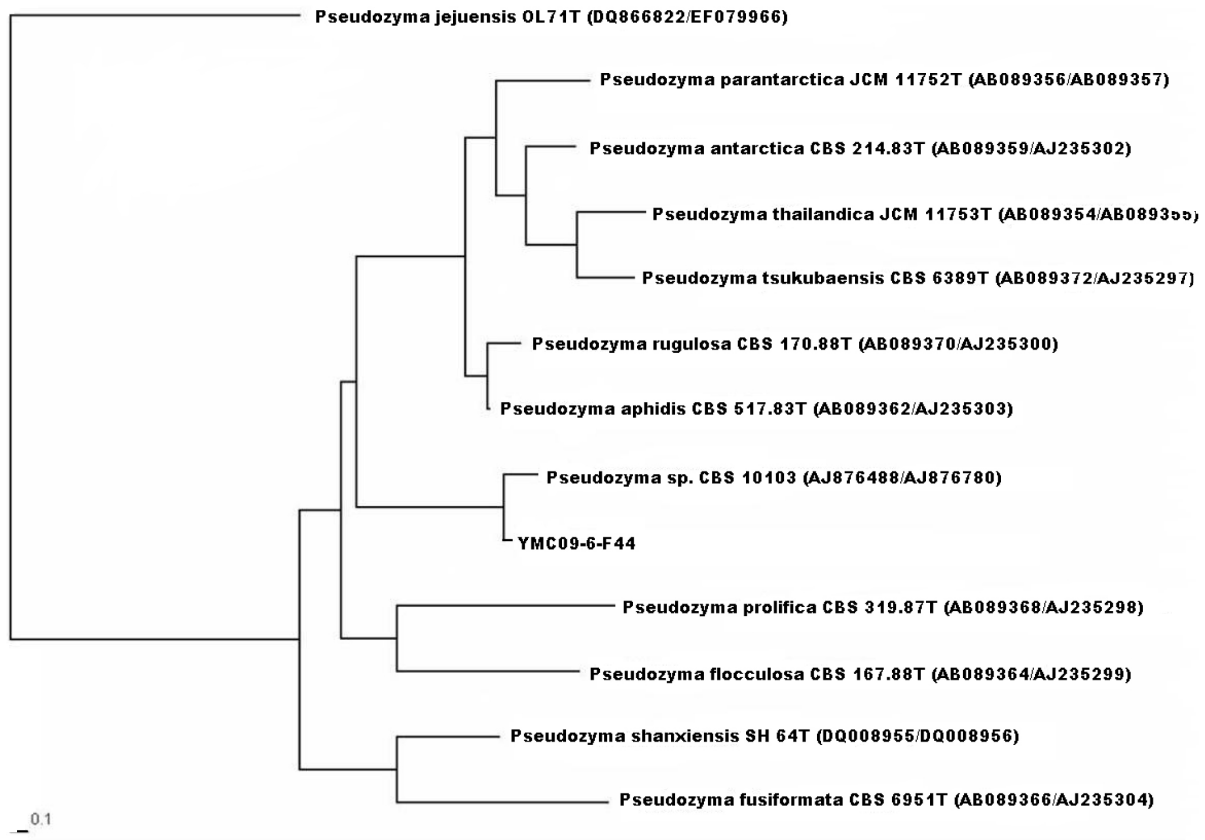

Fig. 2. Molecular phylogenetic tree of strain YCM09/6/F44 and other related reference species derived from the sequence of D1/D2 domain of 26S rRNA. The bar indicates genetic distance.

initiation of antifungal therapy, and on hospital day 86, the patient expired due to multiorgan failure.

DISCUSSION

In 1985, Bandoni [9] established the genus *Pseudozyma* with the species *Pseudozyma prolofica*. Subsequent reports suggested that this genus included more than 10 species with a broad range of phenotypic features such as colony morphology, carbon and nitrogen assimilation, and glucose fermentation patterns [1, 2, 10-13]. Lately, the industrial applications of *Pseudozyma* strains have attracted the attention of biotechnologists [14]. For example, *Pseudozyma flocculosa* produces flocculosin that shows excellent activity against plant pathogenic fungi, and *Pseudozyma antartica* produces glycolipid biosurfactants and lipases that are useful in variable industries [15, 16].

Most *Pseudozyma* sp. has been isolated from plants, except for those indicated in the following 2 reports [1-3]. In the first study, 3 *Pseudozyma* strains, namely, *Pseudozyma parantarctica*, *Pseudozyma thailandica*, and *Pseudozyma antarctica* were isolated from blood of patients with leptospirosis and aseptic meningitis, acute asthmatic attack and respiratory failure, and spontaneous pneumothorax in Thailand. However, the authors did not present their clinical progression of the patients [2]. In the second study, *Pseudozyma aphidis* strain was isolated from a central venous catheter and blood sample of a child with short gut syndrome. The patient had intermittent fevers up to 39.7℃. Initially, yeast and methicillin-resistant coagulase-negative staphylococci were grown from the blood samples obtained through the catheter. Bacteremia was resolved with vancomycin and ceftazidime, but subsequent blood cultures indicated sustained fungemia. Fever was resolved with IV fluconazole treatment after catheter removal [3]. However, there is very limited information

on the clinical significance of *Pseudozyma* strains due to the paucity of reported cases, especially when the strain is isolated from sterile specimens other than blood [2, 3]. In our study, the pathogenicity of the *Pseudozyma* strain remains obscure because of the complicated conditions accompanied by underlying malignancy and co-isolation of MRSA; however, it can be predicted that coinfection with the fungus probably contributed to the worsening of the patient's clinical status.

Interestingly, the species could not be identified by using the YBC card (bioMérieux). There is limited understanding of rare yeasts, including *Pseudozyma* strains, and the improvements in this field have been slow because of the difficult identification procedures [4]. Molecular methods might be very useful for detecting these rare yeasts, including *Pseudozyma* strains [2, 17]. In the CLSI guidelines [17], there were no recommended primers designed specifically to identify *Pseudozyma* species. For the *Ustilago* species, a close relative of *Pseudozyma* species, ITS region is presented as a useful target for species identification [17]. In this study, we sequenced both ITS region and D1/D2 domain, as recommended in previous reports [4, 5], and obtained satisfactory results.

Similar to other related studies [2, 3], we could not identify the infection source in our case. We hypothesized that the fungi probably entered during needle biopsy. The increasing number of immunocompromised patients has broadened the spectrum of yeast-related diseases [2]. Nonetheless, the pathogenicity of yeasts and their association with human diseases are yet to be determined [2, 3]. Therefore, further studies are required to understand the role of pathogenic yeasts. To the best of our knowledge, this is the first report on the isolation of a *Pseudozyma* strain from cerebral abscess and the third one on isolation from a clinical specimen.

REFERENCES

1. Boekhout T and Fell JW. *Pseudozyma* Bandoni emend. Boekhout and a comparison with the yeast state of *Ustilago maydis* (de Candolle) Corda. In: Kurtzman CP and Fell JW, eds. The yeasts: a taxonomic study. 4th ed. Amsterdam: Elsevier Science Publishers, 1998:790-7.
2. Sugita T, Takashima M, Poonwan N, Mekha N, Malaithao K, Thungmuthasawat B, et al. The first isolation of ustilaginomycetous anamorphic yeasts, *Pseudozyma* species, from patients' blood and a description of two new species: *P. parantarctica* and *P. thailandica*. Microbiol Immunol 2003;47:183-90.
3. Lin SS, Pranikoff T, Smith SF, Brandt ME, Gilbert K, Palavecino EL, et al. Central venous catheter infection associated with *Pseudozyma aphidis* in a child with short gut syndrome. J Med Microbiol 2008; 57:516-8.
4. Fell JW, Boekhout T, Fonseca A, Scorzetti G, Statzell-Tallman A. Biodiversity and systematics of basidiomycetous yeasts as determined by large-subunit rDNA D1/D2 domain sequence analysis. Int J Syst Evol Microbiol 2000;50 Pt 3:1351-71.
5. Kurtzman CP and Robnett CJ. Identification of clinically important ascomycetous yeasts based on nucleotide divergence in the 5′ end of the large-subunit (26S) ribosomal DNA gene. J Clin Microbiol 1997; 35:1216-23.
6. Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res 1997;25:4876-82.
7. Saitou N and Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 1987;4:406-25.
8. Felsenstein J. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 1985;39:783-91.
9. Bandoni RJ. On an undescribed, pleomorphic hyphomycete from litter. Bot J Linn Soc 1985;91:37-43.
10. Wei YH, Lee FL, Hsu WH, Chen SR, Chen CC, Wen CY, et al. *Pseudozyma antarctica* in Taiwan: a description based on morphological, physiological and molecular characteristics. Bot Bull Acad Sin 2005; 46:223-9.
11. Wang QM, Jia JH, Bai FY. *Pseudozyma hubeiensis* sp. nov. and *Pseudozyma shanxiensis* sp. nov., novel ustilaginomycetous anamorphic yeast species from plant leaves. Int J Syst Evol Microbiol 2006;56: 289-93.
12. Boekhout T, Fell JW, O'Donnell K. Molecular systematics of some yeast-like anamorphs belonging to the *Ustilaginales* and *Tilletiales*. Stud Mycol 1995;38:175-83.
13. Boekhout T, Bandoni RJ, Fell JW, Kwon-Chung KJ. Discussion of teleomorphic and anamorphic genera of heterobasidiomycetous

yeasts. In: Kurtzman CP and Fell JW, eds. The yeasts: a taxonomic study. 4th ed. Amsterdam: Elsevier Science Publishers, 1998:609-25.
14. Golubev VI. Mycocinogeny in smut yeast-like fungi of the genus *Pseudozyma*. Mikrobiologiia 2007;76:813-6.
15. Mimee B, Labbe C, Pelletier R, Belanger RR. Antifungal activity of flocculosin, a novel glycolipid isolated from *Pseudozyma flocculosa*. Antimicrob Agents Chemother 2005;49:1597-9.
16. Kitamoto D, Isoda H, Nakahara T. Functions and potential applications of glycolipid biosurfactants--from energy-saving materials to gene delivery carriers. J Biosci Bioeng 2002;94:187-201.
17. Petti CA; Clinical and Laboratory Standards Institute. Interpretive criteria for identification of bacteria and fungi by DNA target sequencing: approved guideline. Document MM18-A. Wayne, PA: Clinical and Laboratory Standards Institute, 2008:54-5.