

**A case of bronchial aspergillosis caused by *Aspergillus udagawae* and its  
mycological features**

Hiroshi Gyotoku<sup>1,2</sup>, Koichi Izumikawa<sup>2</sup>, Hideki Ikeda<sup>1</sup>, Takahiro Takazono<sup>2</sup>, Yoshitomo  
Morinaga<sup>3</sup>, Shigeki Nakamura<sup>2</sup>, Yoshifumi Imamura<sup>2</sup>, Tomoya Nishino<sup>2</sup>, Taiga  
Miyazaki<sup>2</sup>, Hiroshi Kakeya<sup>2</sup>, Yoshihiro Yamamoto<sup>2</sup>, Katsunori Yanagihara<sup>3</sup>, Akira  
Yasuoka<sup>4</sup>, Takashi Yaguchi<sup>5</sup>, Hideaki Ohno<sup>6</sup>, Yoshitsugu Miyzaki<sup>6</sup>, Katsuhiko Kamei<sup>5</sup>,  
Tetsuro Kanda<sup>1</sup> and Shigeru Kohno<sup>2</sup>

<sup>1</sup>Internal Medicine, Goto Central Hospital, Goto, Japan

<sup>2</sup>Department of Molecular Microbiology and Immunology Nagasaki University  
Graduate School of Biomedical Sciences, Nagasaki, Japan

<sup>3</sup>Department of Laboratory Medicine, Nagasaki University Hospital, Nagasaki, Japan

<sup>4</sup>Infection Control and Education Center, Nagasaki University Hospital, Nagasaki,  
Japan.

<sup>5</sup>Medical Mycology Research Center, Chiba University, Chiba, Japan

<sup>6</sup>Department of Chemotherapy and Mycoses, National Institute of Infectious Diseases,  
Tokyo, Japan

**Key words:** *Aspergillus udagawae*, diabetes mellitus, bronchial aspergillosis

Corresponding author:

Koichi IZUMIKAWA, M.D., Ph.D.

Department of Molecular Microbiology and Immunology,

Nagasaki University Graduate School of Biomedical Sciences

1-7-1 Sakamoto, Nagasaki 852-8501, JAPAN

Phone: +81-95-819-7273, Fax: +81-95-849-7285

E-mail: [koizumik@nagasaki-u.ac.jp](mailto:koizumik@nagasaki-u.ac.jp)

**Conflicts of interest:**

All authors declare no conflicts of interest regarding this report.

## ABSTRACT

*Aspergillus udagawae* and *A. fumigatus* share similar morphological features but they differ genetically. This is an important clinical distinction, because *A. udagawae* is less sensitive to amphotericin B than *A. fumigatus*. We encountered a rare case of bronchial infection with *A. udagawae* that was successfully treated with voriconazole. An 82-year-old woman with diabetes mellitus complained of blood sputum. Bronchoscopy revealed a white plugged region at the origin of the right bronchi B5. Cytological study revealed a clot of filamentous fungi and *Aspergillus* spp. was detected by culture. Molecular analysis revealed that the causative agent was *A. udagawae*, and voriconazole was used for the treatment. The *A. udagawae* strain isolated in this case was less sensitive to amphotericin B, less virulent in immunosuppressed mice, and more sensitive to hydrogen peroxide, features that are almost identical to those of the previously reported *A. udagawae* strains. We should be aware that the emergence of new *Aspergillus* strains that might pose a clinical threat.

## **INTRODUCTION**

*Aspergillus udagawae* is a fungus that belongs to the genus *Aspergillus* section *Fumigati* and is quite similar to *A. fumigatus* morphologically. Recent studies have shown that *A. udagawae* is genetically dissimilar to *A. fumigatus*, and that *A. udagawae* is less sensitive to amphotericin B [1]. Only a few cases have been reported to date [2-4]. Here, we report the first description of a case of bronchial aspergillosis caused by *A. udagawae* in a patient with mild diabetes mellitus. Since only limited data regarding the mycological features of the clinical isolates of *A. udagawae* is available [5], the results of *in vitro* and *in vivo* experiments regarding drug susceptibility, pathogenicity in mice, and sensitivity to hydrogen peroxide are also presented.

## **CASE REPORT**

An 82-year-old female patient with diabetes mellitus visited Goto Central Hospital complaining of bloody sputum for the previous 2 days. Chest X-ray revealed infiltrates in the right middle lung field and computed tomography (CT) image showed segmental infiltrates in the right middle lobe (Figure 1A). She was immediately admitted for further examination and treatment. On admission, her vital signs were as follows: height,

145 cm; body weight, 51 kg; body temperature, 37.1°C; heart rate, 76 beats/minute with a regular rhythm; blood pressure, 130/64 mmHg; respiratory rate, 12 breaths/minute; and SpO<sub>2</sub>, 97% (room air). Physical examination revealed no rales, murmurs, or signs of systemic lymphadenopathy, hepatosplenomegaly, pretibial edema, and neurological abnormalities. Laboratory findings upon admission were as follows: white blood cell count, 12280/μL with a shift to the left (neutrophils, 80%); C-reactive protein, 0.26 mg/dL; erythrocyte sedimentation rate, 19 mm/h; β-D glucan, 5.4 pg/mL (cut-off, 20 pg/mL); and HbA1c, 6.8%. Test for precipitating antibodies to *Aspergillus* were negative, serum *Aspergillus* galactomannan antigen (ELISA) was positive at 1.4 (cut-off, 0.5) and IgE (RAST) against *Aspergillus* was negative. Bacterial pneumonia was suspected, and therefore sulbactam/ampicillin (4.5 g/day) was initiated. Two days after admission, bronchoscopy revealed a white plug-like polypoid region with a hemorrhagic tendency at the origin of the right bronchi B5 (Figure 2). A biopsy from this region was avoided because of the hemorrhagic tendency, and brushing followed by washing with saline was subsequently performed. Cytological examination of the bronchial lavage fluid revealed a clot of Y-shaped filamentous fungi with septa and microbiological studies detected *Aspergillus* spp. No other microorganisms including bacteria were isolated. Bronchial aspergillosis was diagnosed and the therapy with

antibacterial drugs was changed to 400 mg/day of voriconazole (VRCZ). The bloody sputum gradually improved and disappeared after 6 days of VRCZ treatment. Chest X-ray showed improvement 8 days after VRCZ administration. The patient was discharged on day 24 and oral VRCZ was continued on an outpatient basis. Two weeks after discharge, liver dysfunction was identified by an elevated aspartate aminotransferase, 53 IU/L (normal range: 8-38 IU/L), and VRCZ was changed to 100 mg/day of itraconazole. Treatment was discontinued after 3 months, when infiltrates as observed on chest X-rays had completely disappeared (Figure 1B). The titer of serum *Aspergillus* galactomannan antigen (ELISA) was also decreased to 1.0 at the time of discharge. The patient has remained free of recurrent infection.

## **MYCOLOGICAL FEATURES OF ISOLATED *ASPERGILLUS* SPECIES**

Colonies of the isolate grown on agar were examined by light microscopy and morphologically characterized. Figure 3 shows the appearance of a colony grown on modified Drigalski agar (Eiken Chemical Co., Ltd., Tokyo, Japan) for 7 days at 37°C (A) and the light microscopy findings of lactophenol cotton-blue staining (B). The isolated strain was included in the *Aspergillus* section *Fumigati* based on macro- and micro-morphological characteristics; namely, greenish-white colonies, uniseriate fruiting structures, phialides covering the upper half to two-thirds of the vesicle, and an essentially smooth conidial surface. Molecular techniques were applied for further identification. The genomic DNA of this isolate was prepared using Genterukun® (Takara Bio Inc., Ltd., Otsu, Japan) and the  $\beta$ -tubulin gene was directly sequenced from PCR products using the primer pair Bt2a and Bt2b [6]. The PCR products were sequenced using the BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA, USA) and an ABI PRISM® 3130ABI Genetic Analyzer (Applied Biosystems), according to the manufacturer's instructions. We edited DNA sequences using ATGC Ver. 4 sequence assembly software (Genetyx Co., Tokyo, Japan). The GenBank database at the NCBI website

(<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) was then searched using the  $\beta$ -tubulin gene sequence and the BLAST algorithm. The results of the database search showed that the isolate had 100% similarity to the sequences from *A. udagawae* or its teleomorph (accession numbers AB248294-248297 and DQ058392, respectively), with the number of hits ranging from 453/453 base pairs. The morphological characteristics of the isolate and the results of the BLAST search of the strain were consistent with those of *A. udagawae*. Figure 4 compares the appearance and growth features of the colonies of *A. fumigatus* and *A. udagawae* grown on Czapek-Dox agar after 2 and 7 days at 37 °C and after 7 days at 45 °C. The appearance of *A. udagawae* and *A. fumigatus* differed, and the former was unable to grow at 45 °C, unlike *A. fumigatus*. The antifungal susceptibility of the isolate was also determined retrospectively using the Clinical and Laboratory Standards Institute M38-A2 broth microdilution method [7]. Minimum inhibitory concentrations (MICs) of itraconazole, VRCZ, and amphotericin B and minimum effective concentration (MEC) of micafungin were determined. The MICs of itraconazole, VRCZ and amphotericin B were 0.5, 0.5 and 2  $\mu\text{g/mL}$ , respectively, and the MEC of micafungin was  $\leq 0.015 \mu\text{g/mL}$ . These data compared with epidemiological cutoff values indicated that the *A. udagawae* strain recovered from this patient is not susceptible to amphotericin B [8]. The pathogenesis of the isolate was evaluated using a

mouse model and compared with that of *A. fumigatus* B-5233 strain (kindly provided by Dr. K.J. Kwon-Chung, NIH, Bethesda, MD, USA). Eight-week-old female ICR mice (Charles River Breeding Laboratories, Shiga, Japan) were immunosuppressed by subcutaneous injections of 200 mg/kg of cortisone acetate (Sigma, Tokyo, Japan) on days -1, 0 and 1 and then intratracheally challenged on day 0 with  $5 \times 10^5$  conidia of *A. fumigatus* B-5233 or *A. udagawae*. Survival was monitored as described with minor modification [9] for 14 days after the challenge and data are presented from one representative experiment using groups of 9 mice each. All animal experiments were reviewed and approved by an ethical committee of Nagasaki University Animal Center. Survival curves were generated using the Kaplan-Meier method and statistical differences were evaluated by the log-rank test. Figure 5 shows that *A. udagawae* was statistically less virulent ( $P \leq 0.01$ ) than *A. fumigatus* B-5233. We also examined and compared the sensitivity of the isolated *A. udagawae* strain and *A. fumigatus* B-5233 to hydrogen peroxide, a reactive oxygen species (ROS), by measuring the metabolic activity with the XTT assay as described with minor modification [5]. Briefly, the conidia of *A. fumigatus* B-5233 and *A. udagawae* were incubated with 0-20 mM hydrogen peroxide. The conidia were examined after 20 h and OD<sub>450</sub> was measured. Data from two experiments that included triplicate measurements involving each

concentration and showed that the isolated *A. udagawae* strain was metabolically less active than *A. fumigatus* B-5233; this was statistically significant at the concentration of 5 mM ( $P < 0.05$  by the Student's t-test) (Figure 6). These data indicate that the *A. udagawae* isolated in this case was more sensitive to ROS, which is produced by phagocytes as part of the primary defense system against pathogens.

## DISCUSSION

Advances in molecular techniques and tools have led to the understanding that *A. udagawae* and *A. fumigatus* are different species. The most important clinical feature of *A. udagawae* is that it is less sensitive to amphotericin B compared to *A. fumigatus*. Since many laboratories do not characterize *Aspergillus* spp. beyond morphological diagnosis, the potential prevalence of *A. udagawae* may be underestimated.

To our knowledge, this is the first report of bronchial aspergillosis caused by *A. udagawae*. The underlying diseases among patients infected with *A. udagawae* are chronic granulomatous disease and myelodysplastic syndrome [4]. These diseases usually worsen immunocompromised status, which in turn allows *A. udagawae* to induce invasive aspergillosis with poor prognosis [2-4]. The underlying disease of the patient in this case study was mild diabetes mellitus; this might explain why this *A. udagawae* case was not fatal. One limitation of the diagnosis was that biopsy sample could not be obtained from both of the plugged and the peripheral affected region (end side) due to a high hemorrhagic tendency. The confirmed diagnosis of invasive pulmonary aspergillosis was not made due to lack of pathological evidence indicating invasion of the *Aspergillus* hyphae into tissue, including blood vessels. Biopsy of the

bronchial tissue would have probably confirmed the possible invasion of the bronchial tissue by *A. udagawae*. However, the cytological examination and microbial culture proved that the bronchial plugged region was caused by *A. udagawae* and the diagnosis of bronchial aspergillosis was considered as reasonable. Allergic bronchial pulmonary aspergillosis (ABPA) was another possibility. However, the patient did not have a history of bronchial asthma, eosinophilia, *Aspergillus* antibodies and elevated IgE, which ruled out the diagnosis of ABPA as defined by Rosenberg [10].

Comparison of the mycological features of *A. udagawae* and *A. fumigatus* by Sugui et al. [5] showed that *A. udagawae* is less sensitive to amphotericin B, less virulent and more sensitive to hydrogen peroxide than *A. fumigatus* and their colonies appear different. Furthermore, *A. udagawae* grows more slowly than *A. fumigatus*, with no growth at 45° C. The characteristics of the *A. udagawae* strain isolated in this case were almost identical to these features. The next challenge will be to easily distinguish *A. udagawae* from *A. fumigatus* in the laboratory and clinical setting. We performed further molecular characterization of the isolate because the color of the colony was greenish white and the conidial surface appeared smooth. These findings differ from those of the typical *A. fumigatus*, having blue green colonies and spinose conidia. However, some *A. fumigatus* also may display a greenish white appearance and it is

actually unclear whether their appearance of conidia is smooth or spinose under a light microscope. Hence, molecular characterization was required for definite identification. The difference of maximum growth temperature is most significant and easily applied in the laboratory where molecular tools are unavailable. Furthermore, it is important to spread the awareness that bronchial aspergillosis can be caused by *A. udagawae* and that antifungal drugs other than amphotericin B are more appropriate.

In conclusion, this is the first report of a bronchial *A. udagawae* infection that was successfully treated by VRCZ. The characteristics of the *A. udagawae* strain isolated from this patient were almost identical to those of the known *A. udagawae* strains [5].

## FIGURE LEGENDS

Figure 1. Imaging findings upon admission and after antifungal drug administration.

Chest X-ray shows infiltrates in the right middle lung field and computed tomography image shows segmental infiltrates at the right middle lobe (A). Chest X-rays show absence of infiltrates after 4 months of treatment with antifungal agent (B).

Figure 2. Bronchoscopy findings.

White plug-like polypoid region with hemorrhagic tendency is evident at the origin of the right bronchi B5.

Figure 3. Macroscopic and microscopic appearance of *Aspergillus udagawae* isolate in this case.

Greenish white colonies were observed on modified Drigalski agar after 7 days at 37°C (A). Findings from light microscopy and lactophenol cotton-blue staining (B) magnification ( $\times 400$ ) indicate uniseriate fruiting structures, phialides covering the upper half to two-thirds of the vesicle, and essentially smooth conidia.

Figure 4. Growth characteristics of *Aspergillus fumigatus* and *A. udagawae* on

Czapek-Dox agar compared after 2 and 7 days at 37 °C and after 7 days at 45 °C.

The macroscopic appearance of *A. udagawae* and *A. fumigatus* differs and *A. udagawae* could not grow at 45 °C, unlike *A. fumigatus*.

Figure 5. Survival curves of immunosuppressed mice infected with *Aspergillus fumigatus* and *A. udagawae*.

*A. udagawae* is statistically less virulent ( $P \leq 0.01$ ) than *A. fumigatus* B-5233.

Figure 6. Effect of hydrogen peroxide on *Aspergillus fumigatus* B-5233 and *A. udagawae* as evaluated by XTT assays.

Metabolic activity of *A. udagawae* is significantly reduced compared with that of *A. fumigatus* B-5233.

## REFERENCE

1. Balajee SA, Nickle D, Varga J, Marr KA. Molecular studies reveal frequent misidentification of *Aspergillus fumigatus* by morphotyping. *Eukaryot Cell* 2006; **5**: 1705-1712.
2. Kano R, Itamoto K, Okuda M, *et al.* Isolation of *Aspergillus udagawae* from a fatal case of feline orbital aspergillosis. *Mycoses* 2008; **51**: 360-361.
3. Posteraro B, Mattei R, Trivella F, *et al.* Uncommon *Neosartorya udagawae* fungus as a causative agent of severe corneal infection. *J Clin Microbiol* 2011; **49**: 2357-2360.
4. Vinh DC, Shea YR, Sugui JA, *et al.* Invasive aspergillosis due to *Neosartorya udagawae*. *Clin Infect Dis* 2009; **49**: 102-111.
5. Sugui JA, Vinh DC, Nardone G, *et al.* *Neosartorya udagawae* (*Aspergillus udagawae*), an emerging agent of aspergillosis: how different is it from *Aspergillus fumigatus*? *J Clin Microbiol* 2010; **48**: 220-228.
6. Glass NL, Donaldson GC. Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. *Appl Environ Microbiol* 1995; **61**: 1323-1330.
7. Clinical and Laboratory Standards Institute. Reference method for broth dilution antifungal susceptibility testing of filamentous fungi. 2nd ed.

Approved standard M38-A2. Wayne, PA: Clinical and Laboratory Standards Institute; 2008.

8. Rodriguez-Tudela JL, Alcazar-Fuoli L, Mellado E, *et al.* Epidemiological cutoffs and cross-resistance to azole drugs in *Aspergillus fumigatus*. *Antimicrob Agents Chemother* 2008; **52**: 2468-2472.
9. Takazono T, Izumikawa K, Mihara T, *et al.* Efficacy of combination antifungal therapy with intraperitoneally administered micafungin and aerosolized liposomal amphotericin B against murine invasive pulmonary aspergillosis. *Antimicrob Agents Chemother* 2009; **53**: 3508-3510.
10. Rosenberg M, Patterson R, Mintzer R, *et al.* Clinical and immunologic criteria for the diagnosis of allergic bronchopulmonary aspergillosis. *Ann Intern Med* 1977; **86**: 405-414.

Figure 1. Transitional change of findings of chest X-ray films from admission to discharge

A)



B)



Figure 2. Finding of bronchoscopy



Figure 3. Macroscopic and microscopic appearance of *Aspergillus udagawae* isolate in this case.

A)



B)

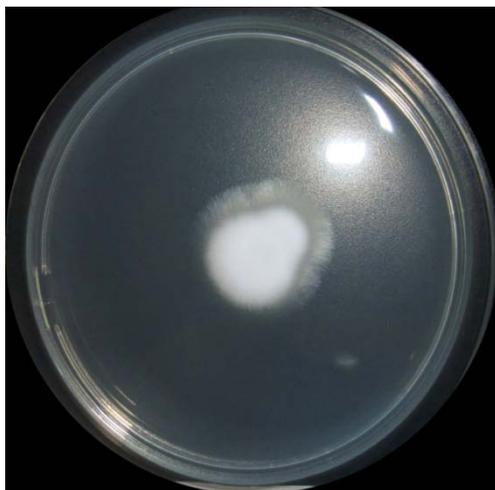


Figure 4. Appearance of colony of *Aspergillus fumigatus* and *A. udagawae* on Czapek-Dox agar at different incubation time and temperature

*A. fumigatus*



*A. udagawae*



2 days at 37°C

7 days at 37°C

7 days at 45°C

Figure 5. Survival curve of *Aspergillus fumigatus* and *A. udagawae* infected mice with immunosuppression

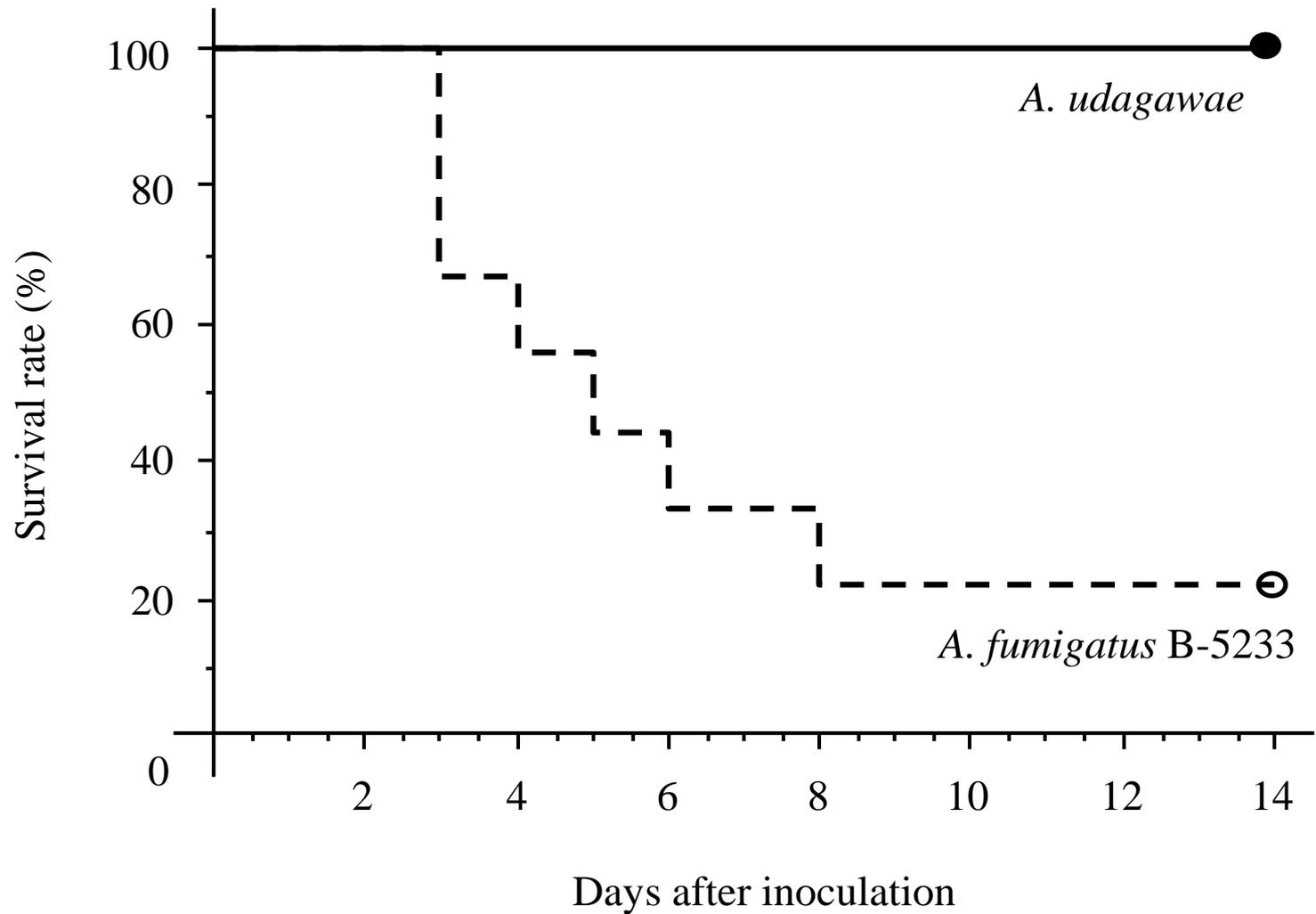
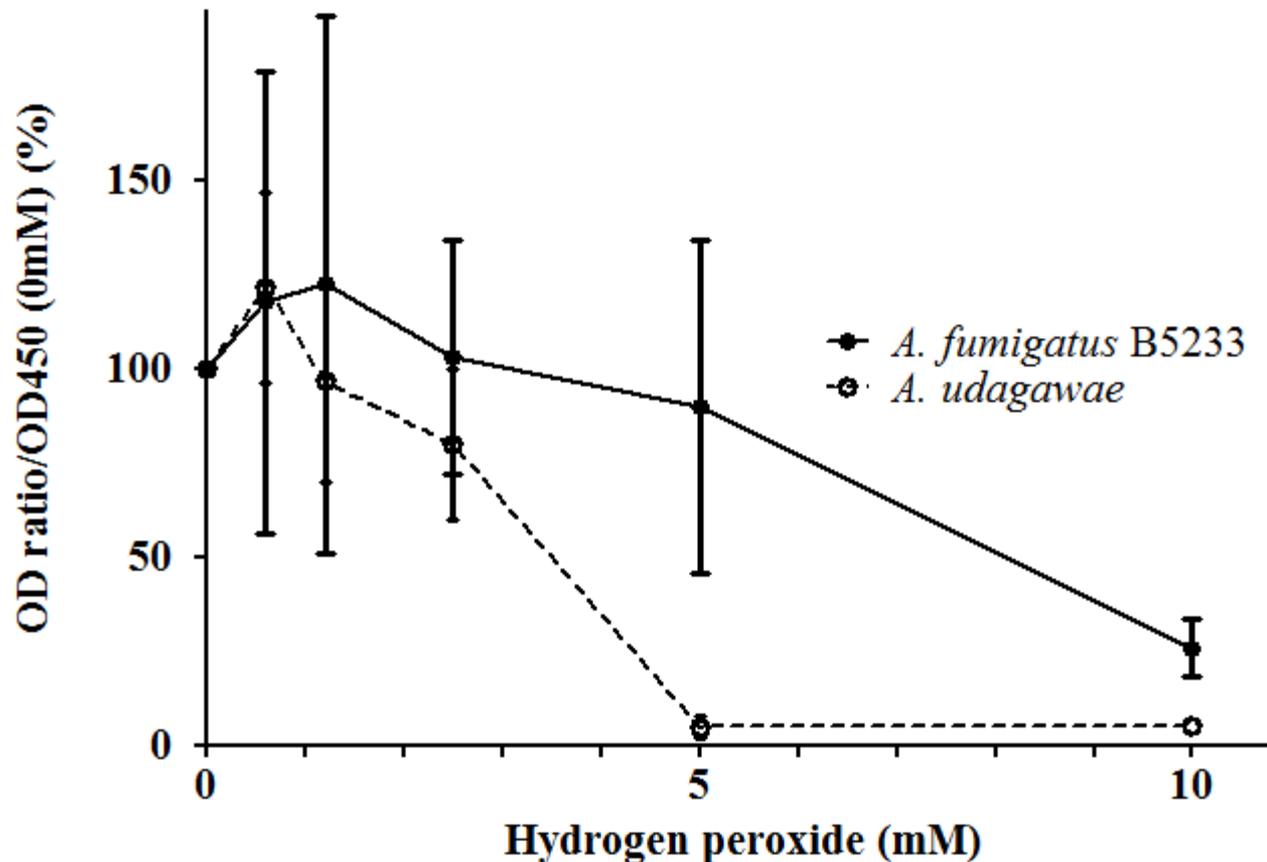


Figure 6. Effect of hydrogen peroxide on *Aspergillus fumigatus* B-5233 and *A. udagawae* evaluated by XTT assay



5mM: P=0.0009 (t-test)