MYCOLOGY

Increased release of glucuronoxylomannan antigen and induced phenotypic changes in *Trichosporon asahii* by repeated passage in mice

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Clinically important fungi such as *Candida albicans* and *Cryptococcus neoformans* are known to undergo phenotypic changes after repeated subculture or passages *in vivo*. However, there are no reports describing this phenomenon in *Trichosporon* species. This study investigated whether *in vivo* passages of environmental isolates of *Trichosporon asahii* in mice changes their phenotype; three environmental isolates and 14 clinical isolates (from deep-seated infections) were used. The shape of the colony and cell type were observed, and the titre of glucuronoxylomannan (GXM) antigen and concentration of (1→3)-β-D-glucan were measured for each isolate. Changes in these features were also examined after three passages of the environmental isolates in mice. The shape of colonies and cell types were clearly different in environmental and clinical isolates. Furthermore, the clinical isolates released significantly higher levels of GXM antigen than environmental isolates (titre: log$_2$ 9.4 SD 0.7 versus log$_2$ 5.4 SD 1.4). The phenotype of passaged isolates was significantly different from the original environmental isolates with respect to the morphology of colonies and cell type and GXM release (titre: log$_2$ 10.0 SD 0.7 versus log$_2$ 5.4 SD 1.4). These results suggest that the phenotypic changes in *T. asahii* occur as a result of *in vivo* passages. This process may allow a proportion of the fungal population to escape eradication by the host immune system, as GXM antigen is considered to protect the fungi against phagocytosis by polymorphonuclear leucocytes and monocytes *in vivo*.

Introduction

*Trichosporon asahii* is one of a number of opportunists mycotic pathogens that can cause life-threatening infections in immunocompromised patients. Deep-seated trichosporonosis is mainly observed in neutropenic patients receiving chemotherapy for haematological malignancies and solid tumours. This systemic disease is associated with morbidity severe conditions, such as progressive respiratory failure, renal failure and disseminated intravascular coagulation syndrome, and is also known to be associated with a poor prognosis [1–4].

*T. beigelii* (*T. cutaneum*) was initially reported as the causative agent of this disease. However, it was reclassified into 20 species by the molecular evolution classification system, based on DNA–DNA homology, introduced by Guého *et al.* in 1992 [5]. According to the new classification, the species most often associated with deep-seated trichosporonosis are *T. asahii* and *T. mucoides* [6]; these are also the major causative agents of the summer-type of hypersensitivity pneumonitis. Thus, it is suggested that these fungi can cause hypersensitivity pneumonitis as allergens and deep-seated trichosporonosis as pathogens, depending on the host's immunological status [7]. It is thought that these fungi enter the body *via* areas where indwelling vascular catheters and drainage tubes are inserted, *via* damaged skin areas in burn patients and by microbial translocation from the intestinal mucosa [8].

Several investigators have reported that clinical isolates of *T. beigelii* (the previous nomenclature included all 20 types of *Trichosporon* spp.) differ from environmental isolates in several phenotypes. Lee *et al.* [9] classified the morphological characteristics of *T. beigelii* and reported that morphologies of cells and
colonies of isolates from deep-seated infection were different from those of environmental and superficial clinical isolates. Lyman et al. [10] also reported that clinical isolates of T. beigelii from deep-seated infection produced more glucuronoxylomannan (GXM) antigen than isolates from environmental sources or superficial infection, and they suggested that these differences might explain their pathogenicity.

In this regard, clinically important fungi such as Candida albicans and Cryptococcus neoformans are known to change their phenotype after repeated subcultures or in-vivo passages [11–16]. This process is thought to allow some fungi to escape eradication by the host immune system [11, 13–15]. However, to our knowledge, there are no studies that have previously examined phenotypic changes within the genus Trichosporon after passages in vivo.

This study compared the phenotypes of environmental isolates and clinical isolates (from deep-seated infections) of T. asahii with respect to morphological features and the release of GXM antigen and (1 → 3)-β-D-glucan. Environmental isolates of T. asahii were also examined quantitatively for phenotypic changes after three passages in a murine host.

Materials and methods

Organisms

T. asahii isolates used in this study are listed in Table 1; there were 3 environmental isolates and 14 clinical isolates (which were stored in the Second Department of Internal Medicine at Oita Medical University). The clinical isolates were obtained from autopsy lungs, blood, urinary catheters, mediastinal drainage fluid, stool, venous catheters, urine and sputum samples. The environmental isolates, which were isolated from the floors of the houses of the patients with summer-type hypersensitivity pneumonitis, were kindly supplied by Teikyo University Research Center for Medical Mycology. All clinical isolates were identified as T. asahii var. asahii on the basis of DNA sequence homology analysis.

Morphological examination

T. asahii isolates, which were stored in skimmed milk suspension at −80°C, were cultured at 37°C for 48 h on Sabouraud Dextrose Agar (SDA; Eiken Chemical Co., Tokyo, Japan). All morphological examinations were performed on colonies grown from pinpoint inoculations on a new SDA plate at 37°C for 7 days. The growth of colonies was monitored macroscopically and classified by the method reported by Lee et al. [9]. T. asahii was inoculated with a platinum wire on to cornmeal medium (Nissui Pharmaceutical Co., Tokyo, Japan) and incubated at 37°C for 72 h. The cell morphology was examined by light microscopy (×400) and classified.

Supernate preparation

The method described by Lyman et al. [10] was used for supernate preparation. Briefly, T. asahii isolates that had been stored in skimmed milk suspension at −80°C were cultured at 37°C for 48 h on SDA, subcultured at 37°C for 48 h on SDA, harvested with a platinum loop and suspended in and washed three times with endotoxin- and (1 → 3)-β-D-glucan-free saline (Otsuka, Tokyo, Japan). In the next step, the T. asahii isolates (1 × 10^6 cfu/ml) were incubated in RPMI 1640 containing 0.025 M N-2-hydroxyethylpiperazine-N’-2-ethane Sulphonic acid (HEPES) buffer and L-glutamine (Gibco BRL, Life Technologies, Tokyo, Japan) for 48 h at 37°C in a water bath with shaking. Immediately after this incubation, the cells were counted with a haemocytometer to determine the final density. The supernates were diluted with RPMI 1640 to prepare a fixed number of 1 × 10^6 cfu/ml so that the amount of antigen produced could be analysed on a per-cell basis. The cells were removed by centrifugation at 1500 g for 10 min. To remove the cells completely, the supernates

**Table 1. Morphology of the environmental isolates and clinical isolates**

<table>
<thead>
<tr>
<th>Isolate no.</th>
<th>Source</th>
<th>Colony</th>
<th>Cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>TIMM1318</td>
<td>Environment (floor)</td>
<td>Rugose</td>
<td>Hyphae</td>
</tr>
<tr>
<td>TIMM1574</td>
<td>Environment (floor)</td>
<td>Rugose</td>
<td>Hyphae</td>
</tr>
<tr>
<td>TIMM1706</td>
<td>Environment (floor)</td>
<td>Rugose</td>
<td>Hyphae</td>
</tr>
<tr>
<td>OU152</td>
<td>Clinical (autopsy lung)</td>
<td>Powdery</td>
<td>Conidia</td>
</tr>
<tr>
<td>OU161</td>
<td>Clinical (autopsy lung)</td>
<td>Powdery</td>
<td>Conidia</td>
</tr>
<tr>
<td>OU239</td>
<td>Clinical (blood)</td>
<td>Powdery</td>
<td>Conidia</td>
</tr>
<tr>
<td>OU93001</td>
<td>Clinical (urinary catheter)</td>
<td>Powdery</td>
<td>Conidia</td>
</tr>
<tr>
<td>NU93002</td>
<td>Clinical (blood)</td>
<td>Powdery</td>
<td>Conidia</td>
</tr>
<tr>
<td>NU93003</td>
<td>Clinical (mediastinal drained fluid)</td>
<td>Powdery</td>
<td>Mixed</td>
</tr>
<tr>
<td>OU94001</td>
<td>Clinical (stool)</td>
<td>Powdery</td>
<td>Conidia</td>
</tr>
<tr>
<td>OU94002</td>
<td>Clinical (venous catheter)</td>
<td>Powdery</td>
<td>Conidia</td>
</tr>
<tr>
<td>OU94003</td>
<td>Clinical (urine)</td>
<td>Powdery</td>
<td>Conidia</td>
</tr>
<tr>
<td>OU94004</td>
<td>Clinical (stool)</td>
<td>Powdery</td>
<td>Conidia</td>
</tr>
<tr>
<td>OU94005</td>
<td>Clinical (urine)</td>
<td>Powdery</td>
<td>Conidia</td>
</tr>
<tr>
<td>OU94006</td>
<td>Clinical (sputum)</td>
<td>Powdery</td>
<td>Conidia</td>
</tr>
<tr>
<td>OU94007</td>
<td>Clinical (urinary catheter)</td>
<td>Powdery</td>
<td>Conidia</td>
</tr>
<tr>
<td>OU94008</td>
<td>Clinical (stool)</td>
<td>Rugose</td>
<td>Conidia</td>
</tr>
</tbody>
</table>

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were filtered with a Millex-GP sterilising filter (0.22-
μm pore size filter; Millipore, Bedford, MA, USA). The samples were stored at −20°C until required for
GXM antigen and (1 → 3)-β-D-glucan assays.

GXM antigen assay
GXM antigen release in the supernates was determined
semi-quantitatively with anticytptococcal cross-reactive
antigen in a latex agglutination test. The assay kit used
in this experiment was Serodirect ‘Eiken’ Cryptococ
coccus® (Eiken Chemical Co.). The assays were
performed according to the manufacturer’s instructions.
Serial two-fold dilutions of each sample were prepared
in RPMI 1640, and the titre was determined from the
endpoint. Each assay was performed three times.

(1 → 3)-β-D-glucan assay
Endotoxin- and (1 → 3)-β-D-glucan-free glassware and
plasticware were used for each assay. Sample was
diluted to 1 in 10 and 1 in 100 with endotoxin- and
(1 → 3)-β-D-glucan-free distilled water (Otsuka). The assays
were performed according to the instructions provided
with the Fungitec G® test kit (G test, Seikagaku
Kogyo, Tokyo, Japan), but omitting the pre-treatment step for
the removal of coagulase in the sample as the sample
was not blood. Subsequently, 50 μl of each diluted
solution were mixed with 50 μl of the main reagent of the
G test kit in each well of a (1 → 3)-β-D-glucan-free 96-
well plate (Toxipet Plate; Seikagaku). After incubation at
37°C for 30 min, 50 μl each of sodium nitrite solution,
ammonium sulphate solution and N-(1-naphthyl) ethyle
nediamine dihydrochloride were added to complete the
diazo coupling reaction. Absorbance of each well was
measured at a wavelength of 545 nm in a spectrophotometer
(Multiscan Multisoft; Labsystems Japan,
Tokyo, Japan). The (1 → 3)-β-D-glucan value was
obtained by comparison with the value for a standard
solution. Each assay was performed three times.

Preparation of passaged isolates
Eight week-old male ICR mice (average weight, 28 SD
4 g; Charles River Japan, Oita, Japan) were used in this
study. Mice were fed dried food designed for experi
ments and, as prophylaxis against concurrent bacterial
infections, they were provided with water containing
vancomycin (VAN, Shionogi, Osaka, Japan) 50 μg/ml
and gentamicin (GEN, Schering-Plough, Osaka, Japan)
10 μg/ml. Each mouse was treated with an intraperi
toneal injection of cyclophosphamide (CPM, Shionogi
Co.) 200 mg/kg/day on days −3 and −2 and predni
solone (PSL, Shionogi) 30 mg/kg/day on day −1 to
induce immunosuppression. Before inoculation, three
environmental isolates of T. asahii were grown at 37°C
for 48 h on SDA and then subcultured on to fresh SDA
and incubated for a further 48 h to ensure purity and
viability. Mature fungi were harvested and suspended
in sterile distilled water and filtered through sterile
gauze to remove the remaining agar and clumps of
organisms. Serial 10-fold dilutions were made of each
suspension in sterile distilled water and the organisms
were counted with a haemocytometer. To confirm the
haemocytometer count, diluted cell suspensions were
cultured on SDA at 37°C for 48 h. The fungal
suspensions were diluted with distilled water and
0.3 ml of the environmental isolate no. TIMM1318
(7.5 × 10⁵ cfu/mouse), 0.3 ml of isolate no. TIMM
1574 (3.7 × 10⁶ cfu/mouse) and 0.3 ml of isolate no.
TIMM1706 (3.0 × 10⁶ cfu/mouse) were individually
injected into mice through the tail vein on day 0. The
inoculum size of each T. asahii isolate was selected so as to
allow a survival rate of 100% in immunocom-
promised mice, as well as allow re-isolation of the
fungi from the kidneys at 2 weeks. Two weeks later, the
infected mice were killed by ether anaesthesia. Both
kidneys were removed surgically and homogenised in
5 ml of sterilised distilled water with a tissue homo
geniser. The suspensions were filtered through sterile
gauze and grown at 37°C for 48 h on SDA, followed by
subculture at 37°C for 48 h on SDA. The fungi
obtained were re-used for murine passage, samples
were also stored in a skimmed milk suspension at
−80°C as the first passaged isolates. After three
passages through mice, the second and third passaged
isolates were prepared. The passaged isolates were
examined morphologically and subjected to GXM
antigen and (1 → 3)-β-D-glucan assays. Each assay
was performed three times. All animal experiments
were performed according to the guidelines of the
Ethical Committee for Animal Experiments at Oita
Medical University.

Statistical analysis
Results of GXM antigen and (1 → 3)-β-D-glucan
assays are expressed as mean and SD. The unpaired
Student’s t test and Welch’s t test were used for
comparison between two groups. A p value of <0.05
was considered significant.

Results
Morphological differences between environmental
and clinical isolates
Results of morphological analysis of colonies and cells
of environmental and clinical isolates are summarised
in Table 1. The colonial morphology was classified
according to the method proposed by Lee et al. [9].
The ‘rugose’ type consisted of white, jagged, peaked
colonies (Fig. 1a), whereas the ‘powdery’ type con
sisted of finely granulated colonies (Fig. 1b). There
were clear differences between the colonial morpho
logy and cell type of environmental and clinical
isolates. Colonies of all environmental isolates were of
the rugose type, whereas all clinical isolates, except for
no. OU94008, were of the powdery type. Isolate
OU94008 was of the rugose type. With regard to the
cell type, environmental isolates consisted of >99% hyphae (Fig. 1c), whereas all clinical isolates except for no. NU93003 consisted of >90% blastoconidia and arthroconidia (Fig. 1d). Isolate NU93003 formed both conidia and hyphae in almost equal amounts and was consequently treated as a mixed pattern (Table 1).

**GXM antigen and (1→3)-β-D-glucan release by environmental and clinical isolates**

Table 2 shows the results of GXM antigen and (1→3)-β-D-glucan assays in environmental and clinical isolates. The mean titre of GXM antigen in three environmental isolates was log₂ 5.4 SD 1.4 (range, log₂ 3.7–log₂ 6.3) and the concentration of released (1→3)-β-D-glucan was 374.3 SD 411.0 pg/ml (range, 79.6–778.5 pg/ml). The mean titre of GXM antigen in 14 strains of clinical isolates was log₂ 9.4 SD 0.7 (range, log₂ 8.3–log₂ 10.3) and the concentration of released (1→3)-β-D-glucan was 59.4 SD 55.8 pg/ml (range, 23.3–153.8 pg/ml). There were no significant differences in the values based on isolation sites among the clinical isolates. The mean titre of GXM antigen released by clinical isolates was significantly higher

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**Fig. 1.** Morphology of environmental and clinical isolates. For macroscopic examination, both isolates were cultured on SDA plates at 37°C for 7 days. (a) A representative environmental isolate (TIMM1706) showing a giant colony of the rugose type. (b) A representative clinical isolate (OMU239), showing a colony of the powdery type. For microscopic examination, both isolates were cultured by slide culture at 37°C for 72 h. (c) A representative environmental isolate (TIMM1706) showing hyphae formation. (d) A representative clinical isolate (OMU239) showing conidia formation (magnification ×400). Bars, 0.1 mm.
than that released by environmental isolates (p < 0.01). In contrast, the mean concentration of (1 → 3)-β-D-glucan released by environmental isolates was significantly higher than that released by clinical isolates (p < 0.01).

**Comparison of morphological features between original and passaged environmental isolates**

Table 3 shows the results of analysis of morphological features of colonies and cells of the original environmental isolates and the same isolates after three passages through mice. With regard to the morphology of colonies, all original environmental isolates were of the rugose type as mentioned above (Fig. 2a), whereas all passaged isolates had changed to the powdery colony type (Fig. 2b). The cell morphology of all environmental isolates consisted of >99% hyphae (Fig. 2c), whereas all passaged environmental isolates except for TIMM1574 consisted of >90% both blastoconidia and arthroconidia (Fig. 2d). Isolate TIMM1574 showed a mixed pattern.

**GXM antigen and (1 → 3)-β-D-glucan release by original and passaged environmental isolates**

As shown in Table 3, the GXM antigen titre was significantly higher in all passaged isolates compared with the original environmental isolates. However, there was no significant difference in (1 → 3)-β-D-
glucan release between each original and passaged isolate. The titre of released GXM antigen and concentration of \((1\rightarrow3)\beta\text{-D-glucan}\) at each passage of the original environmental isolates are shown in Fig. 3. Passage through mice was clearly associated with a progressive increase in GXM antigen release, but there were no clear changes in \((1\rightarrow3)\beta\text{-D-glucan}\) release.

The mean titre of GXM antigen in three passaged environmental isolates was significantly higher than that of the original environmental isolates (log 2 10.0 SD 0.7 versus log 2 5.4 SD 1.4, \(p < 0.01\)). However, there was no clear difference in \((1\rightarrow3)\beta\text{-D-glucan}\) value between the two groups (108.3 SD 99.9 versus 374.3 SD 411.0, NS).

### Discussion

It has been reported that clinical isolates of *T. beigelii* differ from environmental isolates in several phenotypes [9, 10]. Based on these early studies, the present study compared the phenotypes of environmental isolates and clinical isolates of *T. asahii*. The concentration of \((1\rightarrow3)\beta\text{-D-glucan}\) released by environmental isolates was higher than that by clinical isolates. We propose the following explanation for the finding that the environmental isolates released more \((1\rightarrow3)\beta\text{-D-glucan}\) than the clinical isolates. As \((1\rightarrow3)\beta\text{-D-glucan}\) is a polysaccharide forming the major structural component of the fungal cell wall [17, 18], the amount of \((1\rightarrow3)\beta\text{-D-glucan}\) released must depend on the surface area of a cell. The surface area of cells differs depending on whether they are in the form of hyphae or conidia (the surface area of a hypha should be larger than that of a conidium). The environmental isolates of *T. asahii* grow as hyphae whereas the clinical isolates grow as conidia. Therefore, when equal numbers of the environmental isolates and clinical isolates are compared according to the methods of Lyman *et al.* [10], the environmental isolates released more \((1\rightarrow3)\beta\text{-D-glucan}\) into the supernates than clinical isolates.

<table>
<thead>
<tr>
<th>Isolate no.</th>
<th>Colony</th>
<th>Cell</th>
<th>GXM antigen(^a)</th>
<th>((1\rightarrow3)\beta\text{-D-glucan})^1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mean (SD) log(_2) titre</td>
<td>Mean (SD) pg/ml</td>
</tr>
<tr>
<td>Original</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TIMM1318</td>
<td>Rugose</td>
<td>Hyphae</td>
<td>6.3 (0.6)</td>
<td>264.6 (252.7)</td>
</tr>
<tr>
<td>TIMM1574</td>
<td>Rugose</td>
<td>Hyphae</td>
<td>3.7 (0.6)</td>
<td>79.6 (24.1)</td>
</tr>
<tr>
<td>TIMM1706</td>
<td>Rugose</td>
<td>Hyphae</td>
<td>6.3 (0.6)</td>
<td>778.5 (466.6)</td>
</tr>
<tr>
<td>Passaged</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TIMM1318</td>
<td>Powdery</td>
<td>Conidia</td>
<td>9.3 (0.6)^1</td>
<td>193.7 (126.6)</td>
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<tr>
<td>TIMM1574</td>
<td>Powdery</td>
<td>Mixed</td>
<td>10.3 (0.6)^1</td>
<td>101.7 (53.7)</td>
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<tr>
<td>TIMM1706</td>
<td>Powdery</td>
<td>Conidia</td>
<td>10.3 (0.6)^1</td>
<td>29.6 (27.8)</td>
</tr>
</tbody>
</table>

\(^a\)GXM antigen assay was performed three times for each isolate and expressed as mean (SD).

\(^1\)(1\rightarrow3)\beta\text{-D-glucan} assay was performed three times for each isolate and expressed as mean (SD).

### Table 2. Comparison of phenotypes between environmental isolates and clinical isolates

<table>
<thead>
<tr>
<th>Source</th>
<th>Isolate no.</th>
<th>GXM antigen(^a)</th>
<th>((1\rightarrow3)\beta\text{-D-glucan})^1</th>
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</thead>
<tbody>
<tr>
<td>Environmental</td>
<td>TIMM1318</td>
<td>6.3 (0.6)</td>
<td>264.6 (252.7)</td>
</tr>
<tr>
<td></td>
<td>TIMM1574</td>
<td>3.7 (0.6)</td>
<td>79.6 (24.1)</td>
</tr>
<tr>
<td></td>
<td>TIMM1706</td>
<td>6.3 (0.6)</td>
<td>778.5 (466.6)</td>
</tr>
<tr>
<td>Clinical</td>
<td>OU152</td>
<td>10.3 (0.6)</td>
<td>50.4 (7.6)</td>
</tr>
<tr>
<td></td>
<td>OU161</td>
<td>9.3 (0.6)</td>
<td>84.1 (38.3)</td>
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<tr>
<td></td>
<td>OU239</td>
<td>9.7 (0.6)</td>
<td>26.2 (19.7)</td>
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<tr>
<td></td>
<td>OU93001</td>
<td>9.3 (0.6)</td>
<td>34.9 (2.5)</td>
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<td></td>
<td>OU93002</td>
<td>10.3 (0.6)</td>
<td>153.8 (130.7)</td>
</tr>
<tr>
<td></td>
<td>OU93003</td>
<td>8.3 (0.6)</td>
<td>23.3 (4.1)</td>
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<td></td>
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<td>9.0 (0.0)</td>
<td>104.1 (83.5)</td>
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<td></td>
<td>OU94002</td>
<td>9.0 (0.0)</td>
<td>37.9 (31.2)</td>
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<tr>
<td></td>
<td>OU94003</td>
<td>9.0 (0.0)</td>
<td>27.9 (8.5)</td>
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<td>OU94004</td>
<td>10.3 (0.6)</td>
<td>73.8 (79.5)</td>
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<td>OU94005</td>
<td>9.0 (0.0)</td>
<td>60.5 (29.9)</td>
</tr>
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<td>OU94006</td>
<td>9.3 (0.6)</td>
<td>33.8 (16.9)</td>
</tr>
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<td></td>
<td>OU94007</td>
<td>9.3 (0.6)</td>
<td>60.5 (35.5)</td>
</tr>
<tr>
<td></td>
<td>OU94008</td>
<td>9.3 (0.6)</td>
<td>60.7 (51.2)</td>
</tr>
</tbody>
</table>

\(^a\)GXM antigen assay was performed three times for each isolate and expressed as mean (SD).

\(^1\)(1\rightarrow3)\beta\text{-D-glucan} assay was performed three times for each isolate and expressed as mean (SD).
On the other hand, the level of GXM antigen released in the supernates of clinical isolates was higher than in environmental isolates. GXM antigen of *T. beigelii* is an extractable, heat-stable antigen that shares antigenic determinants with glucuronoxylomannan of the capsular polysaccharide of *Crypt. neoformans* [19]. GXM antigen is localised to the cell wall and to fibrillar extracellular matrix projecting from the cell wall of *T. beigelii* [20, 21]. The significantly higher GXM antigen titre in clinical isolates relative to that in environmental isolates is likely to be due to a higher release of GXM antigen from the organisms, as it cannot simply be explained by differences in the cell surface area.

Previous studies have shown that *C. albicans* and *Crypt. neoformans* change their phenotypes when they are subcultured repeatedly or passaged *in vivo* [11–16]. *C. albicans* spontaneously switches the morphology of its colonies into at least seven general phenotypes [16]. *Crypt. neoformans* also changes its phenotype including colony type, capsule size, melanin production, GXM structure, virulence for mice, sterol composition and antifungal susceptibility after passages *in vitro* and *in vivo* [11, 12, 14, 15]. Furthermore, serial *Crypt. neoformans* isolates from the same patients were shown to differ with respect to their ability to survive *in vivo*, virulence in a murine model of cryptococcosis, *in-vitro*

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**Fig. 2.** Morphology of a representative environmental isolate (TIMM1318) before and after three passages in mice. Note the change from a giant rugose colony in the original isolate (a) to a powdery pattern in the passaged isolate (b). Note also formation of hyphae in the original isolate (c) and formation of conidia in the passaged isolate (d) (magnification ×400). Bars, 0.1 mm.
growth rates at 37°C and capsule size [13]. It is thought that environmental adaptation brings about phenotypic variations of the fungus after passages *in vitro* or *in vivo*, especially after passages *in vivo*. Therefore, phenotypic changes may play an important role in the ability of the fungus to escape host defences and establish persistent infection.

To our knowledge, there has been no previous report concerning the phenotypic changes of *Trichosporon* species after passages *in vivo*. This study investigated the phenotypic changes of *T. asahii* due to passages *in vivo*. The results of morphological study suggest that passages *in vivo* in mice change the morphology of environmental isolates to that of clinical isolates.

All isolates showed an increased GXM titre after each passage through the mouse. The capsular GXM polysaccharide from *Crypt. neoformans* is thought to be antiphagocytic to polymorphonuclear leucocytes and, thus, is an important virulence factor for this organism [22–24]. Like *Crypt. neoformans*, *T. beigelii* is also quite resistant to phagocytosis by polymorphonuclear leucocytes and monocytes, and highly resistant compared with *C. albicans* [25, 26]. Although *T. beigelii* is not a capsulate organism, its phylogenetic relationship to *Crypt. neoformans* has been established [27, 28]. The restricted phagocytosis of *T. beigelii* is thought to be related to its surface component, GXM antigen, and a correlation has been reported between GXM antigen production and resistance to killing by polymorpho-

**Fig. 2. (continued)**
nuclear leucocytes and monocytes [25, 26]. Previous studies have shown that GXM antigen is involved in the pathogenicity of T. beigelii and that differences in the production of this antigen between environmental and clinical isolates result in the characteristic differences between them [10]. However, in the present study, passages in vivo were associated with increased release of GXM antigen. The results of the present study suggest that the increased release of GXM antigen is not a result of differences in fungal characteristics, but stems from fungus–host interactions, and is a means of enabling the fungus to escape phagocytosis by polymorphonuclear leucocytes and monocytes in vivo. This process may allow a proportion of the fungal population to escape eradication by the host immune system and establish persistent infection. This process may allow a proportion of the fungal population to escape eradication by the host immune system and establish persistent infection. Thus, brain abscess due to persistent T. beigelii infection has been reported [29]. Clinically, phenotypic changes in Trichosporon as well as in C. albicans and Crypt. neoformans may play an important role in the persistence of infection.

Previous reports suggest that phenotypic variations of C. albicans and Crypt. neoformans are associated with karyotypic changes resulting from micro-evolution [13, 14, 30]. As karyotype analyses were not performed in the present study, it is not possible to say whether the phenotypic changes in T. asahii are a result of micro-evolution or phenotypic adaptation. However, clinical isolates and environmental isolates passed in mice did not demonstrate any morphological conversions after repeated subcultures over a 3-month period at room temperature (data not shown). This is indicative of in-vivo selection of the new phenotypically stable variations.

These results are particularly useful for the identification of T. asahii in clinical microbiology laboratories, because they indicate that the marked morphological variations occurring both macroscopically and microscopically appear to follow certain patterns in clinical isolates and that systemic pathogens differ from environmental isolates in morphological features and release of GXM antigen.

Further studies are necessary to determine whether this process results from karyotypic changes and alters the pathogenicity of the organism. Studies specifically designed to examine the survival rate and persistence of environmental T. asahii isolates in various murine body organs are currently being conducted in this department.

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