Molecular and Biological Differences Among *Ochroconis* Strains Collected from Indoor and Outdoor Environments

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The molecular, morphological, and physiological characters of 55 *Ochroconis* strains collected from indoor and outdoor environments were studied. In Japan, *Ochroconis* species are often found in indoor detergent-rich environments, such as bathrooms and washing machines, and the predominant species have been identified as *O. humicola*, similar to that in other Asian and European countries. Although *Ochroconis* species have rarely been found in outdoor environments such as mountains, forests, and agricultural fields, in the present study, *Ochroconis* strains were specifically isolated from the soils of urban city parks. Phylogenetic analysis conducted using the 28S ribosomal RNA (28S rDNA) gene sequence showed that almost all of the *Ochroconis* strains found in indoor environments (i.e., water supply) were *O. humicola*. Although city parks were often surrounded by residences, more than half of the *Ochroconis* strains collected from the soils of city parks examined in this study were different *Ochroconis* species. The ability to use detergents as nutrients was found in a new genetic group (probably a new species) isolated from the soils of city parks as well as in *O. humicola* and *O. constricta*. *Ochroconis humicola* is assumed to adapt mostly to indoor environments and to penetrate from the outdoors, e.g., soils of urban areas. To elucidate the factors promoting indoor fungal predominance, the ability of using surfactants as nutrients was compared among these three species. Additionally, growth under alkaline and drought conditions, and heat tolerance were examined. Indoor predominance of *O. humicola* compared to that of the other two species was attributed to the ability of using a non-ionic surfactant as nutrient and to growth under alkaline conditions.

Key words: Alkaline condition / City park / Detergent / Drought / Heat tolerance.

INTRODUCTION

*Ochroconis*, as well as *Exophiala*, *Cladophialophora*, and *Phialophora*, which is generally designated as black yeast, is found in animals such as ant, fish, chicken, and human, and in soil, leaf litter, and plant leaf. Therefore, *Ochroconis* was thought to adapt to various environments and distribute widely both in animals and plants (Samerpitak et al., 2014). Moreover, previous studies showed that *Ochroconis* was found not only outdoors but, often, also in indoor locations, especially detergent-rich environments such as bathroom areas and washing machines (Hamada, 2005; Lain and de Hoog, 2010; Samerpitak et al., 2015). *Ochroconis humicola* isolated from detergent-rich environments could use detergents as nutrients, which is unique because surfactants generally inhibit the growth of fungi (Hamada and Abe, 2009). Interestingly, the *Ochroconis* species using detergents as nutrients are not only *O. humicola*. A previous study showed that outdoor species such as *O. constricta* and *Ochroconis* sp. (NH503) could also use non-ion surfactants as nutrients (Abe and Hamada, 2011), suggesting that these three species grew in a water supply environment, in which detergent was often used.

In this study, we investigated why only *O. humicola* penetrated into indoor environments compared to the other two *Ochroconis* species. Originally, *O. humicola* was thought to grow in outdoor environments (e.g., the...
reference strain NBRC32054 was previously collected from leaves. Historically, the use of natural soap in Japanese indoor environments began approximately 100 years ago, whereas non-ion surfactants, which compose synthetic detergents and are used by fungi as nutrients, appeared more recently in households. Accordingly, the predominance of O. humicola in indoor environments is believed to be a contemporary event.

Although Ochroconis was rarely found in mountains, forests, and fields, in a preliminary study, its strains have been detected in outdoor urban areas such as the superficial soils of city parks. Some Ochroconis strains found in urban parks were morphologically and physiologically similar to the indoor species, whereas other strains were similar to the reference species isolated from soils and leaves in wild areas. Especially, two species (i.e., O. constricta and Ochroconis sp. including #503) using detergents as nutrients were found in urban parks in a preliminary study. Nowadays, the soils of city parks in various Asian regions seem to be the hotbed of various Ochroconis species. Therefore, the genetic and physiological characters of these species, which were isolated from more than 30 park soil samples, were examined.

In Japan, city parks located in urban areas are numerous and often surrounded by concrete buildings in residential neighborhoods. Typically, large areas of these parks are open spaces with no grass or planted trees. Because the soils of city parks are not a detergent-rich, the factor promoting the growth of Ochroconis was thought to be something different from surfactants. Since various detergents, including soap, were found in domestic water supply, the pH of which is alkaline, the pH of city park soils was compared to that of the soils of rural areas, forests, or fields. Moreover, it was suggested that the environment in superficial soils of city parks change daily according to atmospheric temperature and humidity. The strains isolated from the soils of city parks were compared with those isolated from indoor water supply and with several reference Ochroconis spp. isolated from non-urban soils. In particular, physiological characters such as optimal pH, heat resistance, and drought tolerance were compared.

In this study, the genus Ochroconis was used in place of Scolecobasidium, according to previously published reports (de Hoog et al., 2000; Samerpitak et al., 2014).

**MATERIALS AND METHODS**

**Sampling of soil**

Indoor samples were collected by swabbing or collecting household water and air samples, as reported in previous studies (Abe and Hamada, 2011). Soil samples were collected from 51 city parks. In each park, 30 g of superficial soil was collected within 1 cm from the surface layer at 4-8 spots. After drying on a newspaper, approximately 2 g of collected soil was suspended into 100 mL of distilled water, using a mixer for 30 s. The suspension was diluted 1:100 and 1:1,000, and inoculated onto 1/4 PDA as corresponding medium (Hamada and Abe, 2010). After cultivation for 7-10 d at 25°C, brown or light brown colonies resembling Ochroconis were isolated for purification and cultured on slant for identification. One Ochroconis strain among two or more strains with similar features was selected from each park. In this study, 31 Ochroconis strains were isolated from outdoor environments; specifically, 29 from the soils of 26 city parks, and 2 from the dust of a soap factory and a hotel (Table 2).

The pH of the soil samples (10 g) in which the fungus was detected was measured following a standard method (Page et al., 1982). For comparison, more than 5 soil samples from each forest or field under study were collected. Prior to pH measurement, 20 mL of distilled water was added to the soil sample, and the suspension was mixed and then stirred for 10 min. The pH was measured with a Piccolo pH meter (Hanna; HI1280) within 2 mo after collecting each soil sample.

**Strains and DNA extraction**

The 55 Ochroconis strains, including 44 strains collected newly in this study, were examined and 25 reference strains, together with their sources and localities, are listed in Table 1. All the 24 indoor samples were collected from different houses in Japan, except for 7 samples that were collected in Europe, China, and Vietnam (Table 1). On the other hand, the 31 outdoor samples collected from urban city areas, including soils of city parks and dust on the buildings, were from Japan and China. After identification as Ochroconis at the genus level according to the morphological characteristics (de Hoog et al., 2000), all strains were subcultured at 25°C for a few wk, using 1/4 potato dextrose agar (PDA) medium. For DNA extraction, a small piece of agar collected from one colony by cutting with sterile pins (5×5 mm) was put in 1 mL of phosphate-buffered saline (PBS). The DNA was extracted and purified as mentioned in the previous paper (Abe and Hamada, 2011).

**Polymerase chain reaction (PCR) and phylogenetic analysis**

Previously, we confirmed the phylogenetic topology using 18S ribosomal RNA (18S rRNA), 28S rDNA, translation elongation factor 1 alpha (tef1), and RNA polymerase B subunit II (rpb2) sequences of indoor strains of O. humicola. Since multiple Ochroconis spp. were identical to each other (Abe and Hamada, 2011),
in the present study, we used 28S rDNA sequences for phylogenetic analysis. The amplification of the nuclear 28S rDNA and its sequence analysis were performed as reported previously (Abe and Hamada, 2011). For phylogenetic analysis, the best nucleotide model K2+G+I was applied to the 28S rDNA dataset and the maximum likelihood tree was constructed by including all gap sites and missing data, using MEGA 6. Support for nodes was estimated by bootstrap analysis with 1,000 replicates. The 28S rDNA sequences obtained in this study are available in the International Nucleotide Sequence Database (GenBank/DDBJ/EMBL) under accession numbers LC187195-LC187202 and LC187232-187235.

**Morphological examination**

For each strain, sample colonies grown on 1/4 PDA medium were collected using a sterilized pin for observation. The color, size, shape, and wall structure and

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**TABLE 1. Ochroconis strains collected from the indoor environments**

| Sample number | Accession number | Species Group | City Prefecture/Country | Latitude | Source | Conidia |
|---------------|------------------|---------------|-------------------------|----------|--------|---------|
|               |                  |               |                         |          |        | Color   |
|               |                  |               |                         |          |        | Shape   |
|               |                  |               |                         |          |        | Size    |
|               |                  |               |                         |          |        | Cell    |
|               |                  |               |                         |          |        | Wall    |
| 1841*         | AB600878         | O. humicola   | Edinburgh               | Great Britain | 56 | bathroom | pale brown | cylindrical | 8 | 2 | smooth |
| 1843*         | AB600879         | O. humicola   | London                  | Great Britain | 51 | bathroom | pale brown | cylindrical | 7 | 2 | smooth |
| 488*          | AB564611         | O. humicola   | Malta                   | Malta      | 35 | bathroom | pale brown | cylindrical | 10 | 2 | smooth |
| 391           |                  | O. humicola   | Osaka                   | Osaka      | 35 | air-borne | pale brown | cylindrical | 8 | 2 | smooth |
| 8071*         | AB564609         | O. humicola   | Osaka                   | Osaka      | 35 | WM       | pale brown | cylindrical | 10 | 2 | smooth |
| 782           | AB564612         | O. humicola   | Osaka                   | Osaka      | 35 | WM       | pale brown | cylindrical | 8 | 2 | smooth |
| 673*          | AB564610         | O. humicola   | Osaka                   | Osaka      | 35 | WM       | pale brown | cylindrical | 9 | 2 | smooth |
| 9105*         | AB564613         | O. humicola   | Osaka                   | Osaka      | 35 | bathroom | pale brown | cylindrical | 8 | 2 | smooth |
| 674           |                  | O. humicola   | Osaka                   | Osaka      | 35 | WM       | pale brown | cylindrical | 8 | 2 | smooth |
| 652           |                  | O. humicola   | Osaka                   | Osaka      | 35 | WM       | pale brown | cylindrical | 6 | 2 | verruculose |
| 749           |                  | O. humicola   | Miyakonojo              | Miyazaki   | 31 | bathroom | pale brown | cylindrical | 8 | 2 | smooth |
| 992           |                  | O. humicola   | Naha                    | Okinawa    | 26 | bathroom | pale brown | cylindrical | 9 | 2 | smooth |
| 67            |                  | O. humicola   | Hanoi                   | Vietnam    | 21 | bathroom | pale brown | cylindrical | 7 | 2 | verruculose |
| 693           |                  | O. humicola   | Hanoi                   | Vietnam    | 20 | bathroom | pale brown | cylindrical | 7 | 2 | verruculose |
| 737           |                  | O. humicola   | Brussel                 | Belgium    | 51 | bathroom | pale brown | cylindrical | 7 | 2 | smooth |
| 797           | LC187195         | O. humicola   | Osaka                   | Osaka      | 35 | WM       | pale brown | cylindrical | 10 | 2 | smooth |
| 792*          | AB564614         | O. humicola   | Osaka                   | Osaka      | 35 | bathroom | pale brown | cylindrical | 8 | 2 | smooth |
| 558           |                  | O. humicola   | Osaka                   | Osaka      | 35 | WM       | pale brown | cylindrical | 7 | 2 | verruculose |
| 539*          | AB564615         | O. humicola   | Osaka                   | Osaka      | 35 | bathroom | pale brown | cylindrical | 6 | 2 | smooth |
| 279*          | AB564616         | O. humicola   | Osaka                   | Osaka      | 35 | bathroom | pale brown | cylindrical | 8 | 2 | smooth |
| 517           |                  | O. humicola   | Osaka                   | Osaka      | 35 | dishwasher | pale brown | cylindrical | 8 | 2 | smooth |
| 332           | LC187197         | O. humicola   | Guangzhou               | China      | 23 | bathroom | pale brown | oblong | 9 | 2 | smooth |
| 454           |                  | Ochroconis sp. P | Osaka                   | Osaka      | 35 | dishwasher | pale brown | cylindrical | 8 | 2 | verruculose |
| 523           | LC187200         | Ochroconis sp. P | Osaka                   | Osaka      | 35 | dishwasher | subhyaline | cylindrical | 9 | 2 | smooth |

Reference

NBRC32054 AB564618 O. humicola Israel leaves pale brown cylindrical 9 2 smooth
NBRC39375 AB564619 O. constricta unknown dead leaf pale brown cylindrical 8 2 verruculose
NBRC30094 S. variabile unknown soil pale brown cylindrical 11 2 verruculose
NBRC30095 O. verruculosa unknown soil pale brown oblong 8 2 echinulate
CBS.437.64 O. gallopava USA brain abscess pale brown clavate 14 2 smooth

Sampling site and characteristic of conidia were shown.
The asterisk was added to the strain examined previously.
WM indicates washing machine.
Samples were collected in bathrooms by swab, from washing water in WM or dishwashers, air-borne by air sampler.
Growth of *Ochroconis* spp. on medium including surfactants

Fifteen indoor strains, 24 outdoor strains, and 8 reference strains were examined on the physiological characteristics. The strains with various shape and from various collection sites and with various shape were selected in this study.
The colony size was compared with that on 1/4 PDA, and assessed as follows: ++; same, +; smaller, −; undetectable.

| Sample number | Species      | Group | City           | Source          | 1/4PDA colony number | SO (sodium fatty acid) | AE (non-ion surfactant) |
|---------------|--------------|-------|----------------|------------------|-----------------------|------------------------|-------------------------|
| 391           | O. humicola  |       | Osaka          | air-borne        | 80                    | + 8                    | 0 + 66 + 26             |
| 8071          | O. humicola  |       | Osaka          | WM               | 87                    | + 22                   | 0 + 36 + 8              |
| 468           | O. humicola  |       | Malta          | bathroom         | 51                    | + 34                   | 9 + 9 − 0               |
| 1843          | O. humicola  |       | London         | bathroom         | 60                    | + 35                   | 13 + 23 + 19            |
| 992           | O. humicola  |       | Okinawa        | bathroom         | 55                    | + 20                   | 9 + 8 + 3               |
| 67            | O. humicola  |       | Hanoi          | bathroom         | 52                    | + 34                   | − 0 − 0 − 0             |
| 693           | O. humicola  |       | Hiroshima      | bathroom         | 56                    | + 40                   | − 0 − 0 − 0             |
| 332           | O. humicola  |       | Guangzhou      | bathroom         | 72                    | + 74                   | − 0 + 34 + 21           |
| 797           | O. humicola  |       | Osaka          | WM               | 89                    | + 44                   | + 10 + 90 + 70          |
| 792           | O. humicola  |       | Osaka          | bathroom         | 62                    | + 66                   | + 13 + 16 − 0           |
| 737           | O. humicola  |       | Brussel        | bathroom         | 60                    | + 42                   | − 0 + 30 − 0            |
| 558           | O. humicola  |       | Osaka          | WM               | 52                    | + 26                   | + 14 + 15 − 0           |
| 517           | O. humicola  |       | Osaka          | dishwasher       | 60                    | + 6                    | − 0 + 3 − 0             |
| 454           | Ochroconis sp. | P   | Osaka          | dishwasher       | 62                    | + 70                   | − 0 − 0 − 0             |
| 523           | Ochroconis sp. |     | Osaka          | dishwasher       | 53                    | + 6                    | − 0 − 0 − 0             |
| 508           | O. humicola  |       | Naha           | park             | 78                    | + 74                   | + 80 + 68 + 47          |
| 348           | O. humicola  |       | Guangzhou      | park             | 83                    | + 84                   | + 11 + 26 − 0           |
| 503           | Ochroconis sp. | P   | Higashi-Osaka  | wall             | 77                    | + 65                   | + 21 + 20 − 0           |
| 8652          | Ochroconis sp. | P   | Kitakyushu     | park             | 95                    | + 80                   | + 10 + 82 + 73          |
| 650           | Ochroconis sp. | P   | Akita          | park             | 84                    | + 37                   | − 0 − 0 − 0             |
| 1253          | Ochroconis sp. | P   | Tokyo          | park             | 84                    | + 80                   | − 0 − 0 − 0             |
| 403           | Ochroconis sp. | P   | Tokyo          | park             | 57                    | + 49                   | + 45 − 0 − 0            |
| 1242          | Ochroconis sp. | P   | Nagoya         | park             | 52                    | + 44                   | + 24 + 36 − 0           |
| 1177          | Ochroconis sp. | P   | Kyoto          | park             | 89                    | + 82                   | + 55 − 0 − 0            |
| 1192          | Ochroconis sp. | P   | Ashiya         | park             | 70                    | + 65                   | + 60 − 0 − 0            |
| 26            | Ochroconis sp. | P   | Osaka          | park             | 93                    | + 76                   | + 18 − 0 − 0            |
| 344           | Ochroconis sp. | P   | Osaka          | park             | 94                    | + 87                   | − 0 − 0 − 0             |
| 387           | Ochroconis sp. | P   | Osaka          | park             | 57                    | + 37                   | − 0 − 0 − 0             |
| 3             | Ochroconis sp. | P   | Osaka          | park             | 60                    | + 50                   | + 32 − 0 − 0            |
| 1275          | Ochroconis sp. | P   | Okayama        | park             | 75                    | + 78                   | + 63 − 0 − 0            |
| 372           | Ochroconis sp. | P   | Kagoshima      | park             | 53                    | + 47                   | + 49 − 0 − 0            |
| 5682          | Ochroconis sp. | −   | Naha           | park             | 66                    | + 70                   | − 0 − 0 − 0             |
| 79            | Ochroconis sp. | −   | Kagoshima      | park             | 78                    | − 0                    | − 0 − 0 − 0             |
| 1188          | Ochroconis sp. | −   | Kobe           | park             | 67                    | + 51                   | − 0 − 0 − 0             |
| 11772         | Ochroconis sp. | −   | Kobe           | park             | 92                    | − 0                    | − 0 − 0 − 0             |
| 11543         | O. verruculosa |      | Kyoto          | park             | 96                    | + 94                   | − 0 − 0 − 0             |
| 1234          | O. constricta |      | Nagoya         | park             | 54                    | + 52                   | − 0 + 31 − 0            |
| 1256          | O. tshawytsha |      | Tokyo          | park             | 76                    | + 80                   | − 0 − 0 − 0             |
| 365           | O. tshawytsha |      | Osaka          | park             | 99                    | + 93                   | − 0 − 0 − 0             |

**TABLE 3.** The growth of *Ochroconis* strains collected from the indoor and outdoor environments cultured on the medium of surfactant.

Reference

| Country | Source |
|---------|--------|
| Israel  | leaf   |
| Japan   | unknown |
| Unknown | dead leaf |
| Unknown | soil |
| Unknown | soil |
| Unknown | dead leaf |
| Unknown | litter |
| USA     | brain abscess |

The count and the size of fungal colony on various medium were compared with that on 1/4 PDA medium. The colony size was compared with that on 1/4 PDA, and assessed as follows: ++; same, +; smaller, −; undetectable.
TABLE 4. The growth of *Ochroconis* strains collected from the indoor and outdoor environments cultured under various conditions

| Sample number | Species       | Group      | City       | Source       | Incubation for 10 minutes |
|---------------|---------------|------------|------------|--------------|---------------------------|
|               |               |            |            |              | 1/4PDA colony number | pH9.7 colony number | DG18 colony number | 52°C colony number | 50°C colony number |
| 391           | *O. humicola* | Osaka      | air-borne  | 80 ++ 62     |                         |                         |                         | 0               | 0               |
| 8071          | *O. humicola* | Osaka      | WM         | 87 ++ 65 + 77 |                         |                         |                         | 0 + 6            |
| 468           | *O. humicola* | Malta      | bathroom  | 51 + 50 + 28 + 5 |                         |                         |                         |                 |
| 1843          | *O. humicola* | London     | bathroom  | 60 + 50 + 44 |                         |                         |                         | 0 + 11           |
| 992           | *O. humicola* | Naha       | bathroom  | 55 + 36 + 44 |                         |                         |                         | 0 + 7            |
| 67            | *O. humicola* | Hanoi      | bathroom  | 52 ++ 44 + 39 |                         |                         |                         | 0 ++ 32          |
| 693           | *O. humicola* | Hiroshima  | bathroom  | 56 ++ 58 + 38 + 20 |                         |                         |                         |                 |
| 332           | *O. humicola* | Guangzhou  | bathroom  | 72 + 72 + 67 + 7 |                         |                         |                         | ++ 68            |
| 797           | *O. humicola* | Osaka      | WM         | 89 ++ 75 + 38 + 15 |                         |                         |                         | ++ 68            |
| 792           | *O. humicola* | Osaka      | WM         | 62 ++ 64 + 58 + 2 |                         |                         |                         | + 60             |
| 737           | *O. humicola* | Brussel    | bathroom  | 60 ++ 54 + 60 + 16 |                         |                         |                         | ++ 48            |
| 558           | *O. humicola* | Osaka      | WM         | 52 + 48 + 52 + 3 |                         |                         |                         | + 30             |
| 517           | *O. humicola* | Osaka      | dishwasher | 60 + 37 + 44 |                         |                         |                         | 0 + 2            |
| 454           | *Ochroconis*  | Osaka      | dishwasher | 62 ++ 60 + 55 + 2 |                         |                         |                         | + 57             |
| 523           | *Ochroconis*  | Osaka      | dishwasher | 53 + 19 + 31 |                         |                         |                         | 0               |
| 908           | *O. humicola* | Naha       | park       | 78 + 76 + 78 |                         |                         |                         | 0 + 51           |
| 348           | *O. humicola* | Guangzhou  | park       | 83 ++ 78 + 49 |                         |                         |                         | 0 ++ 14          |
| 503           | *Ochroconis*  | P          | Kitakyushu | 95 + 76 + 35 |                         |                         |                         | 0 ++ 47          |
| 8652          | *Ochroconis*  | P          | Akita      | 84 + 78 + 42 + 2 |                         |                         |                         | ++ 60            |
| 650           | *Ochroconis*  | P          | Tokyo      | 84 + 66 + 56 + 5 |                         |                         |                         | + 64             |
| 1253          | *Ochroconis*  | P          | Tokyo      | 57 + 50 |                         |                         |                         | 0 + 4             |
| 403           | *Ochroconis*  | P          | Nagoya     | 52 + 53 + 48 + 1 |                         |                         |                         | ++ 73            |
| 1177          | *Ochroconis*  | P          | Kyoto      | 89 + 80 + 90 + 3 |                         |                         |                         | ++ 67            |
| 1192          | *Ochroconis*  | P          | Ashiya     | 70 + 70 + 50 |                         |                         |                         | 0 + 42            |
| 26            | *Ochroconis*  | P          | Osaka      | 93 ++ 80 + 87 |                         |                         |                         | 0 + 53            |
| 344           | *Ochroconis*  | P          | Osaka      | 94 + 98 + 71 + 10 |                         |                         |                         | + 94             |
| 387           | *Ochroconis*  | P          | Osaka      | 57 + 48 + 33 + 3 |                         |                         |                         | + 24             |
| 3             | *Ochroconis*  | P          | Osaka      | 60 ++ 51 + 33 |                         |                         |                         | 0 + 28            |
| 1275          | *Ochroconis*  | P          | Okayama    | 75 + 78 + 77 + 2 |                         |                         |                         | + 19             |
| 372           | *Ochroconis*  | P          | Kagoshima  | 53 + 50 + 31 |                         |                         |                         | 0 + 7             |
| 5862          | *Ochroconis*  | –          | Naha       | 66 + 58 + 70 |                         |                         |                         | 0 + 17            |
| 79            | *Ochroconis*  | –          | Kagoshima  | 78 – 0 – 0 |                         |                         |                         | 0 – 0             |
| 1188          | *Ochroconis*  | –          | Kobe       | 67 – 0 – 0 |                         |                         |                         | 0 – 0             |
| 11772         | *Ochroconis*  | –          | Kyoto      | 92 – 0 – 0 |                         |                         |                         | 0 – 0             |
| 11543         | *O. verruculosa* | Kyoto   | park       | 96 + 96 |                         |                         |                         | ++ 69 + 92 |
| 1234          | *O. constricta* | Nagoya | park       | 54 – 0 |                         |                         |                         | ++ 48 + 49 |
| 1256          | *O. ishawyclipsis* | Tokyo | park       | 76 + 34 + 60 |                         |                         |                         | + 31 + 63 |
| 385           | *O. ishawyclipsis* | Osaka | park       | 99 + 90 + 43 |                         |                         |                         | 3 + 34            |

The count and the size of fungal colony on various culture condition were compared with that on 1/4 PDA medium. The colony size was compared with that on 1/4 PDA, and assessed as follows: ++: same, +: smaller, −: undetectable.
In accordance with a previous study (Hamada and Abe, 2009), after pre-culture of the colonies of sample strains and reference strains, 0.5 mL of the conidial suspension from each strain was plated uniformly on two or more culture plates containing media with different compositions (see below). All the plates were incubated at 25±1°C for 10-12 d in an incubator. The fungal growth on medium including two surfactants: sodium oleate (SO), which is the main component of soap; and the non-ionic surfactant polyoxyethylene-(9)-lauryl ether, which is classified as alkyl ether (AE) and an ingredient of synthetic detergents. The media used to test the fungal growth in the presence of different surfactants were as follows: SO or AE both at either 0.1% w/v and 0.5% w/v was added to 15 g/L of Bacto agar (Difco, USA). For emphasizing the effect of surfactant to fungi, both surfactants were used in double concentration, compared to a previous study (Hamada and Abe, 2009). All inoculates were incubated at 25°C for 12-16 d. The growth of the fungi in the presence of the two surfactants at two different dilution levels was compared in terms of colony count and size.

Growth of Ochroconis spp. on other media and pre-incubation

Each strain that grew on the alkaline 1/4 PDA medium was further tested for its tolerance to alkaline conditions. To adjust the pH value of the medium to an alkaline level, a modified version of the method reported by Nagai et al. (1998) was applied. Namely, Na₂CO₃ (3.0 g) and NaH₂PO₄ (3 g) were added to 1/4 PDA. At 25°C, the pH of 1/4 PDA was 6.6, whereas that of the modified (i.e., alkaline) 1/4 PDA was 9.7. The strains were incubated at 25°C for 12-16 d.

To examine the tolerance of the fungi to drought, each strain was cultured on dichloran glycerol (DG18) agar base (Oxoid, England) containing 31.5 g/L and 220 g/L glycerol, with the latter concentration being used to detect xerophilic fungi. The strains were incubated at 25°C in the dark for 12-16 d.

To examine heat stress tolerance of each strain, conidial suspensions of each fungus were placed for 10 min in water baths (Masuda; M-205) at 50°C and 52°C. The temperature deviation was within 1.0°C. After treatment, the suspensions were inoculated onto 1/4 PDA and cultured at 25°C in the dark for 10-12 d.

RESULTS

Molecular characterization of the strains

The 28S rDNA sequences were obtained from all strains from indoor or outdoor urban environments. As shown in Fig.1, the 12 strains from indoors (i.e., bathroom, washing machine, and dishwasher) and the 2 strains (NH908, NH348) from outdoors (park) were classified into the O. humicola group, including the reference strain of O. humicola and the other indoor strains of O. humicola identified in previous studies, indicating that the 14 strains isolated in this study from indoor and outdoor environments are O. humicola. The 20 strains isolated in this study from city parks, one strain (NH503) previously identified in a soap factory, and one indoor strain (NH454) identified in this study from dishwasher were grouped together (park group: Group P); interestingly, they were different from the other Ochroconis species isolated in this study. Among the other 9 strains from outdoors (NH1234, NH11772, NH1188, NH11543, NH385, NH1256, NH5862, NH79) and indoors (NH523), NH1234 was identified as O. constricta, NH11543 as O. verruculosa, and NH1256 and NH385 as O. tshawytschae, which was confirmed by the morphological analysis of the conidia of these 4 strains (see below). Moreover, the strains NH523 from dishwasher, and NH1188 and NH11772 from city parks were distinct from the other Ochroconis species in this group. Moreover, the strains NH79 and NH5862 were closely related with O. bacilliformis and O. anellii, respectively.

Morphological characterization of the strains

When observed under light microscope, the conidia of all the strains in O. humicola isolated from indoors and outdoors were two-celled, pale brown, oblong or cylindrical-shaped, approximately 6-10 µm in length, and smooth-walled or verruculose (Table 1 and Fig.2A), and similar to the conidia of the reference strain NBRC32054, and of NH348 and NH908, which were collected in the city parks. However, the color of the conidia of the indoor strain NH523 was subhyaline, which is different from that of other indoor strains (Fig.2B). The morphological differentiation between NH523 and O. humicola group was confirmed by phylogenetic analysis.

From a morphological viewpoint, the conidia of the predominant strains collected from outdoor urban areas (Group P) were clavate or cylindrical-shaped, two-celled, pale brown, and approximately 7-10 µm in length (Table 2 and Fig.2A). Large variations in the conidia walls of Group P, which included the NH454 strain collected indoors, were observed. The conidia wall was smooth in NH403, whereas it was verruculose in NH454 and verrucose in NH1192. Moreover, the conidia of both NH503 and NH8652 were 6-7 µm in length and echinulate-walled (Fig.2A).

Some strains different from O. humicola and Group P
were also found in the soils of city parks (Table 2). The NH11543 strain showed two-celled, oblong, and sharply echinulate-walled conidia, which resembled those of O. verruculosa, to which NH11543 is also genetically related (Fig.2B). Moreover, the conidia of NH1234 were two-celled, oblong, verrucose, and similar to those of O. constricta, to which NH1234 is genetically related. The conidia of NH1188 and NH11772, which were not genetically identical with previously reported species, were subhyaline, two-celled, and smooth walled. The conidia of the strains NH385, NH1256, NH5862, and NH79 were two-to-four-celled, which was different from those of the two-celled stains from outdoors (Fig.2B). The conidia of NH385 and NH1256 were pale brown and verrucose, similar to the conidia of O. tshawytschae, whereas the conidia of NH79 and NH5862 were not identical to the conidia of any species previously reported.

**Comparison of the growth of Ochroconis spp.**

Most strains isolated from indoors and outdoors grew on 0.1% SO medium regardless of the genetic group.

**FIG. 1.** Phylogenetic relations of 55 Ochroconis strains from indoor and outdoor environments (in Bold) to reference strains of Ochroconis spp. (in Regular), as inferred based on results of distance-based analysis of 28S rDNA sequences. The number of strain and accession was shown in parentheses, and the asterisk was added to the strain examined previously. Bootstrap support (>50% for 1000 replicates) is shown at each node.
NH1234, which were isolated from urban outdoor areas, grew on the 0.1% AE medium. On the 1/4 PDA medium adjusted to pH 9.7, the growth of the strains isolated from indoors and outdoors was compared with that on normal 1/4 PDA medium (Table 4). All of O. humicola and Group P strains grew on alkaline medium. However, 4 out of the 9 strains belonging to any group did not grow on the alkaline medium, including O. constricta NH1234. Additionally, 4 out of 8 reference strains did not grow on alkaline medium, such as the reference strain O. constricta NBRC9357.

The colony size of 7 strains of O. humicola on alkaline medium was similar to that on normal medium, whereas this was true for only 3 strains belonging to Group P. The growth rate of O. humicola on alkaline medium was higher than that of Group P strains. Interestingly, the growth of all of the 3 strains of Group P that use detergents as nutrients (i.e., NH503, 8652, and 1242) decreased under alkaline conditions.

The drought tolerance of indoor and outdoor strains of each species was compared. Most strains of O. humicola and Group P grew on the DG18 xerophilic medium, although the colony size of all the strains examined was smaller than that on the normal medium. Only 4 out of 9 strains (i.e., NH523, NH5862, NH1256, and NH385)
**TABLE 5.** The pH of soil sample of the city park in which *Ochroconis* strain was collected

| Sample number | Group | Park name | City | Latitude | Source | Sample | pH (min-max) | pH |
|---------------|-------|-----------|------|----------|--------|--------|--------------|----|
| 908           | O. humicola | Wakasa | Naha | 26 | park | soil | 7.78 |
| 348           | O. humicola | Jinan Univ. | Guangzhou | 23 | park | soil | 7.32 |
| 503           | *Ochroconis* sp. | Soap factory | Higashi-Osaka | 35 | wall dust | | – ^* |
| 8652          | *Ochroconis* sp. | P | Kokura | Kitakyushu | 34 | park | soil | 7.94 |
| 650           | *Ochroconis* sp. | P | Numata | Akita | 40 | park | soil | 6.56 |
| 1253          | *Ochroconis* sp. | P | Kyobashi | Tokyo | 36 | park | soil | 7.69 |
| 403           | *Ochroconis* sp. | P | Shinagawa | Tokyo | 36 | park | soil | 7.76 |
| 1242          | *Ochroconis* sp. | P | Kameshima | Nagoya | 35 | park | soil | 6.38 |
| 1238          | *Ochroconis* sp. | P | Nortake | Nagoya | 35 | park | soil | 6.49 |
| 1152          | *Ochroconis* sp. | P | Mibuhinoki | Kyoto | 35 | park | soil | 7.22 |
| 1180          | *Ochroconis* sp. | P | Tonoda | Kyoto | 35 | park | soil | 7.78 |
| 1177          | *Ochroconis* sp. | P | Hokodate | Kyoto | 35 | park | soil | 7.35 |
| 1192          | *Ochroconis* sp. | P | Miyazaki | Ashiya | 35 | park | soil | 6.98 |
| 26            | *Ochroconis* sp. | P | Hanahaku | Osaka | 35 | park | soil | 6.37 |
| 344           | *Ochroconis* sp. | P | Utsubo | Osaka | 35 | park | soil | 6.20 |
| 387           | *Ochroconis* sp. | P | Ikutama | Osaka | 35 | park | soil | 6.52 |
| 365           | *Ochroconis* sp. | P | Nagaike | Osaka | 35 | park | soil | 6.39 |
| 3             | *Ochroconis* sp. | P | Nagai | Osaka | 35 | park | soil | 6.40 |
| 12733         | *Ochroconis* sp. | P | Nodacho | Okayama | 35 | park | soil | 6.04 |
| 1275          | *Ochroconis* sp. | P | Nanpo | Okayama | 35 | park | soil | 6.06 |
| 338           | *Ochroconis* sp. | P | Beppu | Miyazaki | 31 | park | soil | 6.13 |
| 511           | *Ochroconis* sp. | P | Hotel | Miyakonojo | 31 | roof dust | | 6.20 |
| 372           | *Ochroconis* sp. | P | Izumi | Kagoshima | 31 | park | soil | 5.68 |
| 79            | *Ochroconis* sp. | | Tenmonkan | Kagoshima | 31 | park | soil | 6.52 |
| 5862          | *Ochroconis* sp. | | | | | | | |
| 1256          | O. tshawytschae | | Kyobashi | Tokyo | 36 | park | soil | 7.85 |
| 385           | O. tshawytschae | | Sanadayama | Osaka | 35 | park | soil | 7.17 |
| 1188          | *Ochroconis* sp. | | Hanakuma | Kobe | 35 | park | soil | 7.57 |
| 11772         | *Ochroconis* sp. | | | | | | | |
| 11543         | O. verruculosa | | Mibuhinoki | Kyoto | 35 | park | soil | 6.34 |
| 1234          | O. constricta | | Makino | Nagoya | 35 | park | soil | 6.56 |

The pH of soil collected from forest and field as control

| Location | Prefecture | Latitude | Location | Source | pH (min-max) | Average pH |
|----------|------------|----------|----------|--------|--------------|------------|
| Hida City | Gifu | 36 | field | soil | 5.26-6.28 | 5.77 |
| Daisen Mt. | Tottori | 35 | forest | soil | 5.25-6.10 | 5.65 |
| Hira Mt. | Shiga | 35 | forest | soil | 5.18-5.69 | 5.48 |
| Daimonnji Mt. | | | forest | soil | 4.79-5.78 | 5.24 |
| Kongo Mt. | Osaka | 34 | forest | soil | 4.67-6.26 | 5.55 |

The asterisk indicates that pH was not measured, because sample dust was not remaining.
that did not belong to the two species grew on DG18 medium.

The heat tolerance of the strains was analyzed according to the incubation temperature (i.e., 50, 52°C) (Table 4). Generally, heat caused the reduction of both colony size and count. At 52°C, a few strains survived, although their size and colony count were smaller than those at room temperature. Half of the strains (15/30) from both O. humicola and Group P (i.e., indoor and outdoor environments, respectively) could grow at 52°C, with 7 and 8 strains belonging to O. humicola and Group P, respectively. Similarly, 4 out of 9 strains not belonging to any group also grew at 52°C. Most strains of O. humicola and Group P survived at 50°C. Among the strains not belonging to any group, 4 strains (i.e., NH523, NH79, NH11772, and NH1188) did not grow at 50°C. Ochroconis humicola and Group P strains showed similar physiological responses to drought and heat tolerance.

The pH of each soil sampled in city parks from which Ochroconis strains were isolated (Table 5) was analyzed. The pH ranged from 5.68 to 7.99, and approximately 43% (13/30) of the strains were found at pH higher than 7.01. The pH of the soils of city parks seems to differ according to cities. Namely, alkaline soil was found in Tokyo and Kyoto City, and slightly acidic soil was found in Osaka and Nagoya City. However, these pH values are comparably more alkaline than that of the soils of forests and fields (Table 5). Specifically, the average pH of the soil in wild areas, which were used as the control, was 5.24-5.77.

**DISCUSSION**

As shown in Figure 1, O. musae, which was recently isolated from bananas (Hao et al., 2013), was classified in the O. humicola group. Since the dimensions of the conidia structures of O. musae were in the range of the morphological variations (wall structure and size of conidia) found in the O. humicola strains examined in the present study, O. musae might be a synonym of O. humicola.

In a previous study, the strain NH503, which was collected from the wall of a soap factory, was genetically different from O. humicola (Fig.1). In this study, 20 predominant strains collected in city parks showed conidia with wall structures varying widely from smooth to verrucose and to echinulate (Table 2). Although the morphological variation among the strains was found, these strains were genetically identified and classified into Group P as a potential new species.

The genetic characteristics highlighted by phylogenetic analysis show that the fungal flora of Ochroconis isolated from indoor and outdoor environments is quite different, although the geographical distance between the collection sites (i.e., households and city parks) was often limited. In the absence of large obstacles, conidia of Ochroconis could immigrate from outdoor to indoor and vice versa, thus mixing with each other. Many air-borne conidia belonging to Group P are thought to exist indoors, although one of those (NH454) was found in a dishwasher (Table 1). However, the species predominant in outdoor urban areas (i.e., Group P) were different from O. humicola, which was collected from houses (Fig.1). This result suggests that Group P and O. humicola could survive outdoors and indoors, respectively, better than they do in other environments.

The growth of predominant Ochroconis strains both from indoors and from city parks was similar under different experimental conditions such as drought, and heat stress (Table 4). However, predominant indoor and outdoor strains behaved differently from each other in the presence of surfactants. As mentioned in a previous study, O. humicola strains could use detergents as nutrients (Abe and Hamada, 2011), whereas outdoor strains such as the Group P did not show the same tendency (Table 3). Although 3 strains of Group P could survive in the presence of 0.1% non-ionic surfactant (AE), 13 and 7 O. humicola strains grew on the medium containing 0.1% or 0.5% AE (Table 3), respectively, which is equivalent to approximately 6 or 30 times the concentration of commercial detergent solutions used for washing clothes, respectively. Therefore, O. humicola strains could use accumulated and condensed detergent inside washing machines. This difference in the physiological characters is thought to affect the distribution of the two Ochroconis species.

Moreover, O. constricta as well as Group P strains from outdoors could survive in the presence of non-ionic surfactant (Table 3), but could tolerate alkaline conditions less than O. humicola (Table 4). Therefore, both abilities to use detergents as nutrients and to grow under alkaline condition were thought to contribute to the survival of O. humicola in indoor environments.

In a previous study, 10 strains isolated from washing machines and bathrooms from Japan and Europe were identified as O. humicola (Abe and Hamada, 2011). In this study, 12 O. humicola strains were obtained from water supply, dishwashers, and bathrooms of Asian countries. Moreover, 2 strains of O. humicola were found in the soils of city parks in Naha, Japan, and Guangzhou, China, which are both located south of parallel 30N. These results imply that O. humicola found in indoor environments using hot water may distribute originally in the soils of warm area all the year, e.g., sub-tropical area. Since non-ionic surfactants were introduced in Japan in washing and cleaning indoor environments approxi-
mately 50 years ago, their presence is recent. Therefore, *Ochroconis* species growing in city parks or other outdoor environments are thought to have immigrated into indoor environments not earlier than 50 years ago.

The number of species growing in the soils of city parks was higher than that in indoor environments, and some of those species were already collected from various wild environments (Table 2). The physiological characters such as drought and heat tolerance of Group P strains were different from those of other strains, which are minor species in city parks. Since city parks were built no longer than 100 years ago, it was proposed that Group P strains adapted to the environment of city parks recently.

Why does *Ochroconis* commonly colonize in city parks? Most *Ochroconis* strains grew on 0.1% soap medium (Table 3). Because the physiological ability to use soap did not particularly benefit *Ochroconis* strains in the parks, another characteristic was considered responsible for the growth of *Ochroconis* outdoors. We assumed that many strains, including those of Group P, were isolated from city parks because they adapted to the pH of those soils. Although the pH of soil is usually acidic, the soils of the city parks analyzed in this study ranged from neutral to slightly acidic pH, which is more alkaline (i.e., less acidic) than those from forests and fields (Table 5), the reason being that the dust of concrete buildings accumulates on the urban soil causing alkaline pH (Wang and Sakamoto, 1994). In this study, neutral or slightly acidic conditions promoted the growth of some *Ochroconis* strains, including Group P strains, whereas 4 out of 9 strains not assigned to any group (i.e., outdoor strains) could grow on alkaline medium (Table 4).

Xerophilic or heat tolerance characteristics are crucial for *Ochroconis* strains to survive in the soils of city parks, which easily dry and become hot during summer compared with forests. Interestingly, a few *Ochroconis* strains, i.e., Group P and *O. tshawytschae*, seem to adapt to dry and heat conditions. However, NH79, NH1188, and NH11772, which are hydrophilic and heat-sensitive, could not grow on alkaline medium. These *Ochroconis* strains have the ability to grow in the soils of city parks because the planted trees and grasses offer a different environment compared to that found in the wild. Therefore, strains growing in city parks are thought to vary physiologically, molecularly, and morphologically. According to this study, the soils of urban city parks seemed to be the hotbed of *Ochroconis* sp., which is predominant in indoor environments, urban areas, and wild forests.

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