Isolation of fungi from a fungivorous insect, the minute brown scavenger beetle (Latridiidae), and their potential ability for mycotoxin production

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Abstract

Fungivorous insects are serious hazard as potential contaminants in food. This study investigated fungi and their mycotoxins isolated from fungivorous insects (minute brown scavenger beetles [Latridiidae]) captured in a building located in Chiba, Narashino. We trapped 18 insects (heads) and isolated 780 colonies of fungi from them, which we classified into 3 genera: Penicillium (90.1%), Aspergillus (7.7%), Cladosporium (1.0%) and others (1.2%). The population of these fungi reflected that of fungi isolated from the environment inhabited by the insects. In the genus Aspergillus, sterigmatocystin and cyclopiazonic acid were detected by a simultaneous detection system of liquid chromatography-quadrupole time-of-flight mass spectrometry when the isolates were cultured in medium. These findings strongly suggest that Latridiidae are not only a physical but also a microbial hazard as a vector for the spread of mycotoxin-producing fungi in factories for food products and the storage of cereals.

Keywords

Fungivorous insect; Minute brown scavenger beetle (Latridiidae); Mycotoxin

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Latridiidae inhabiting a building located in Narashino, Chiba Prefecture, Japan and analyzed the mycotoxins obtained when isolates were cultured in two different types of medium for three weeks.

Latridiidae samples were captured by sticky traps (PP trap; Ikari Shodoku, Co., Ltd., Tokyo, Japan) at five indoor points in a building of Narashino City in Chiba Prefecture, Japan, over a one-month period in the summer of 2016. A total of 18 insects captured by 5 traps were used in this study. Each individual insect was put into a tube containing 0.1 mL sterile phosphate-buffered saline, then homogenized and vortexed for 1 min. The slurry was cultured on a potato dextrose agar plate at 25±1 °C for 7 days. The indoor fungi were collected by the swab method from the floor near where the Latridiidae were trapped. The fungi were counted and morphologically classified into three genera (Aspergillus, Penicillium, Cladosporium) or “others” based on the colony textures and microscopic observations.

Fig. 2(A) shows the genus distribution for the 780 fungal isolates from 18 Latridiidae. The predominant genus was Penicillium, which accounted for more than 90% of all of the isolates, followed by Aspergillus (7.7%), Cladosporium (1.0%) and others (1.2%). Fig. 2(B) shows the genus distribution for the fungi isolated from indoor areas. Here as well, the genus Penicillium was predominant (89.2%), followed by Aspergillus (6.5%), Cladosporium (1.6%) and others (2.7%). These results suggest that the population of isolates from the Latridiidae reflected that of isolates from other indoor areas and show that the species isolated from Latridiidae captured in the buildings preferred Penicillium to Aspergillus, consistent with the report of Hartley and McHugh.

Regarding the genera Penicillium and Aspergillus, isolates were classified into eight and six groups based on the morphological characteristics, respectively. Depending on the number of strains belonging to the group, one to four strains were selected from each group at random, and the selected strain was characterized based on nucleotide sequence similarities of β-tubulin gene, 28S rDNA D1/D2 region and ITS1 region, using nucleotide Basic Local Alignment Search Tool (BLAST) of National Center for Biotechnology Information.

The mycotoxin productions of the selected strains were examined using two kinds of medium (yeast extract sucrose broth [YES] and Czapek yeast autolysate broth [CYB]) and at two temperatures (25±1 °C and 30±1 °C) for three weeks. CYB has the following formulation: Difco™ Czapek-Dox Broth (BD, Franklin Lakes, NJ, USA), 35 g and yeast extract (Merck Millipore, Darmstadt, Germany), 5 g /L distilled water. YES has the following formulation: yeast extract (Merck Millipore), 20 g and sucrose (Kishida Chemical Co., Ltd., Osaka, Japan), 150 g /L distilled water. Multi-mycotoxins qualitative surveillance was performed with liquid chromatography-quadrupole time-of-flight mass spectrometry (Agilent Technologies, Santa Clara, CA, USA). In brief, 5 mL of the cultured medium was extracted with an equal volume of chloroform. After sonication for 20 min, the chloroform layer was collected and evaporated. The extract was resolved with 1 mL of methanol and applied to a liquid chromatography system (Agilent 1290 Infinity II LC system, Agilent Technologies, Waldborn, Germany) with a 100 mm ×2.1 mm i.d. column packed with 1.8 µm ZORBAX Eclipse Plus C18 (Agilent Technologies, Santa Clara, CA, USA) at 40 °C. The liquid chromatography mobile phase was aqueous 0.1% formic acid (A) and methanol (B). The initial condition was 90% A and 10% B, changing linearly to 0% A and 100% B over 30 min. The flow rate was 0.2 mL/min, and the injection volume was 1 µL. Mass spectrometry was performed using a quadrupole time-of-flight mass spec-
trometry system equipped with a dual spray AJS ion source (Agilent 6545 Quadrupole Time-of-Flight LC/MS; Agilent Technologies, Santa Clara, CA, USA) in positive-ion mode with a mass ranging from 100 to 1000 Da and a data rate of 1.5 mass spectra/sec. The instrument acquired data using the following parameters: drying gas temperature, 350 °C; drying gas flow, 10 L/min; nebulizer pressure, 50 psi; sheath gas temperature, 400 °C; sheath gas flow, 12 L/min; VCap. 4000 V; nozzle, 0 V. Under these conditions, as major mycotoxins, aflatoxins, ochratoxin A, citrinin, sterigmatocystin (STE), cyclopiazonic acid (CPA), zearalenone, T-2 toxin and HT-2 toxin could be detected, with a limit of detection of 1 ng/mL.

In Table 1 and 2, the species to which each fungal group showed the most similarity (100% or 99%) in nucleotide BLAST are listed. As shown in Table 1, the fungi classified into group 1 (Test strain No. 1-4) were identified as *A. tubingensis*, those into group 2 (Test strain No. 5-8) as *A. flavus* or *A. oryzae*, those into group 3 (Test strain No. 9-12) as *A. westerdijkiae* or *A. ochraceus*, those into groups 4 (Test strain No. 13, colony color: white) and 5 (Test strain No. 14, colony color: white with a yellow-green center) as *A. protuberus* or *A. versicolor*, and those into group 6 (Test strain No. 15) as *A. sydowii*. Mycotoxins were detected in groups 2, 4, and 5; Sample No. 6-8 of group 2, suspected of being *A. flavus* or *A. oryzae*, produced CPA, and Sample No. 13 and 14 of groups 4 and 5, respectively, suspected of being *A. protuberus* or *A. versicolor*, produced STE. We obtained qualitative data, and the limit of detection of those mycotoxins in the present study was 1 ng/mL. CPA and STE are known to be produced by *A. flavus* and *A. versicolor*, respectively Suppl. 10, 11. While *A. flavus* and *A. westerdijkiae* have been known to produce aflatoxins and ochratoxin A, respectively Suppl. 12, 13, these mycotoxins were not detected in the selected *Aspergillus* strains in this study.

### Table 1 Characterization of the genus *Aspergillus* isolated from Latridiidae and their mycotoxin production

| Isolated fungi Group | Species showing the most similarity (100%) in nucleotide BLAST | Test strain № | Mycotoxinsa | Temperature |
|----------------------|---------------------------------------------------------------|---------------|-------------|-------------|
|                      | β- tubulin | D1/D2 | ITS |
|                      |           |      |     |                    | Mediumb                        | 25 °C | 30 °C |
| group 1              |           |      |     |                    |                                |       |       |
| *A. tubingensis*     | A. heteromorphus | A. niger | A. tubingensis | 1-4 | CYB - -          |       |       |
|                      | A. niger   |      |     |                    | YES - -                        |       |       |
|                      | A. phoenicis |      |     |                    |                                |       |       |
|                      | A. tubingensis |      |     |                    |                                |       |       |
| group 2              |           |      |     |                    |                                |       |       |
| *A. flavus*          | A. bombycis | A. flavus | A. flavus | 5 | CYB - -          |       |       |
| *A. oryzae*          | A. flavus | A. minisclerotigenes | A. oryzae | YES - - |                           |       |       |
|                      | A. oryzae |      |     |                    |                                |       |       |
|                      | A. parasiticus |      |     |                    |                                |       |       |
| group 3              |           |      |     |                    |                                |       |       |
| *A. ochraceus*       | A. westerdijkiae | A. ochraceus* | A. ochraceus* | 9-12 | CYB - -          |       |       |
|                      | A. westerdijkiae |      |     |                    | YES - -                        |       |       |
| group 4              |           |      |     |                    |                                |       |       |
| *A. protuberus*      | A. protuberus | A. versicolor | A. versicolor | 13 | CYB STE          |       |       |
|                      | A. versicolor |      |     |                    | YES STE                        |       |       |
| group 5              |           |      |     |                    |                                |       |       |
| *A. protuberus*      | A. amoenum | A. austroafricanus | A. versicolor | 14 | CYB STE STE |       |       |
|                      | A. protuberus |      |     |                    | YES STE STE                    |       |       |
|                      | A. tabacinus |      |     |                    |                                |       |       |
|                      | A. versicolor |      |     |                    |                                |       |       |
| group 6              |           |      |     |                    |                                |       |       |
| *A. sydowii*         | A. sydowii | A. sydowii | A. versicolor | 15 | CYB - -          |       |       |
|                      | A. sydowii | A. sydowii |              | YES - - |                           |       |       |

a Nucleotide sequence identity: 99%
b YES: yeast extract sucrose broth, CYB: Czapek yeast autolysate broth
c CPA: cyclopiazonic acid, STE: sterigmatocystin
In Table 2, the groups of the genus *Penicillium* isolated from *Latridiidae* are listed. The fungi classified into group 1 (Test strain No. 17-19) were identified as possibly *P. implicatum*, that into group 2 (Test strain No. 20) as possibly *P. chermesinum*, those into group 3 (Test strain No. 21-22) as possibly *P. waksmanii* or *P. corylophilum*, that into group 4 (Test strain No. 23) as possibly *P. rugulosum*, that into group 5 (Test strain No. 24) as *P. fellutanum*, those into group 6 (Test strain No. 25-26) as possibly *P. variabile*, those into group 7 (Test strain No. 27-29) as *P. chrysogenum*, *P. rolfsii* or *P. rubens* and those into group 8 (Test strain No. 31-32) as *P. canescens*. Mycotoxins were not detected in the medium cultured with the selected listed in Table 2.

These results indicated that the strains selected from the genus *Aspergillus* obtained from the beetles possessed the potential to produce the screened mycotoxins in this study, while those from the genus *Penicillium* had no such potential.

Regarding the toxicity of mycotoxins for insects, STE and CPA have been reported to be cytotoxic and anti-insect mycotoxins\(^\text{14,15}\) and are known to be mutagenic mycotoxins for both humans and animals\(^\text{16,17}\). Rohls et al. reported that fungivorous insects avoid feeding on toxic fungi\(^\text{10}\). Given that the isolates in this study were obtained from a homogenized sample, the fungi with the potential ability to produce mycotoxins might have been on the surface of the beetles, rather than in their digestive tract. To explore this hypothesis, further studies focusing on the fungi isolated from the digestive tract of these beetles are needed.

Although the capture area was limited, we found that *Latridiidae* mainly possessed *Penicillium* and *Aspergillus* fungi. A qualitative analysis of multiple mycotoxins revealed that the genus *Aspergillus* had the ability to produce CPA and STE, while the genus *Penicillium* produced no screened mycotoxins in this study on the culture of the selected strains. Vega et al. found that insect parasitoids carried the ochratoxin-producing fungus, *A. westerdijkiae*. The fungus is known for contaminating coffee berry. They suggested that insects might play a role in the dissemination of ochratoxin-
producing fungi. Similar to insect parasitoids, the present results strongly suggest that fungivorous insects, such as Latridiidae, would also be carriers of mycotoxin-producing fungi and spread those fungi to food industrial areas and storage facilities for cereals.

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