Culture condition-dependent metabolite profiling of *Aspergillus fumigatus* with antifungal activity

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**Abstract**

Three sections of *Aspergillus* (five species, 21 strains) were classified according to culture medium-dependent and time-dependent secondary metabolite profile-based chemotaxonomy. Secondary metabolites were analysed by liquid chromatography–electrospray ionisation tandem mass spectrometry (LC–ESI-MS–MS) and multivariate statistical methods. From the *Aspergillus* sections that were cultured on malt extract agar (MEA) and Czapek yeast extract agar (CYA) for 7, 12, and 16 d, *Aspergillus* sections *Fumigati* (*A. fumigatus*), *Nigri* (*A. niger*), and *Flavi* (*A. flavus*, *A. oryzae*, and *A. sojae*) clustered separately on the basis of the results of the secondary metabolite analyses at 16 d regardless of culture medium. Based on orthogonal projection to latent structures discriminant analysis by partial least squares discriminant analysis (PLS-DA), we identified the secondary metabolites that helped differentiate sections between *A. fumigatus* and *Aspergillus* section *Flavi* to be gliotoxin G, fumigatin oxide, fumigatin, pseurotin A or D, fumiquinazoline D, fumagillin, helvolic acid, 1,2-dihydrohelvolic acid, and 5,8-dihydroxy-9,12-octadecadienoic acid (5,8-diHODE). Among these compounds, fumagillin, helvolic acid, and 1,2-dihydrohelvolic acid of *A. fumigatus* showed antifungal activities against *Malassezia furfur*, which is lipophilic yeast that causes epidermal skin disorders.

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**Introduction**

*Aspergillus* is one of the most common soil fungi and is widely distributed all over the world. Approximately 250 *Aspergillus* species descriptions have been published, and the number of species has been increasing as new species are discovered and classification concepts change (Geiser et al. 2007). *Aspergillus* is very important in clinical settings as an opportunistic pathogen and in genetics as a eukaryotic model organism. The species plays a crucial role in food and feed safety because of its production of mycotoxins (Klich 2006). *Aspergillus fumigatus* (section *Fumigati*), *Aspergillus niger* (section *Nigri*), and *Aspergillus flavus* (section *Flavi*) are known to produce very harmful mycotoxins. Hence, these *Aspergillus* species are disease-causing opportunistic pathogens for *humans* and *animals*. On the other hand, *Aspergillus* species, including *A. oryzae*, *A. sojae*, and *A. flavus* (section *Flavi*), as well as *A. niger* (section *Nigri*), are widely used for *fermented* food production such as soy sauce, rice wine, Pu-erh tea, and cheese (Scott & Sullivan 2008; Ku et al. 2009).

Molecular, morphological, and physiological characteristics are considered for traditional *Aspergillus* taxonomic assignment. However, solely using one taxonomic method is not sufficient for *Aspergillus* classification. Recently, combined
identification methods have been used as a polyphasic identification approach to improve the clarity of the classification (Hong et al. 2005; Samson et al. 2006). Also, Meijer et al. (2011) reported the species identification of A. niger and other black aspergillas based on growth and hydrolase profiles. As an alternative method, several studies on chemotaxonomy of different fungi have been reported with different approaches (Ceiser et al. 2007; Frisvad et al. 2008; Kang et al. 2011; Kim et al. 2012). However, secondary metabolite production of fungi was influenced by the culture conditions (medium, incubation time) (Frisvad et al. 2007; Nielsen et al. 2009). The production of several secondary metabolites, which have important activities, such as mycotoxins, were reported by different environments of growth (Boudra & Morgavi 2005; Senyuva 2008). However, information about the incubation condition-dependent impact of total chemotaxonomic features of Aspergillus is scarce.

Liquid chromatography–mass spectrometry (LC–MS) could be applied to the basic detection of chemical substances or biological materials because of the high sensitivity and specificity of the method (Druzhinina et al. 2005; Degenkolb et al. 2008). Principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA) are statistical methods used to determine which metabolites represent differences among species. Therefore, the combination of analytic devices and statistical approaches can be a suitable and powerful tool for classifying microorganisms on the basis of metabolite profiling (Soderstrom & Frisvad 1984; Almassi et al. 1994).

It was reported that Aspergillus spp. possessed antifungal activity against pathogenic microorganisms (Liu et al. 2004). Malassezia furfur is a yeast that causes skin disorders such as dandruff, seborrhoic dermatitis, and tinea versicolour in humans (Marcon et al. 1987). Antifungal activity against M. furfur was observed in other microorganisms (Miranda et al. 2007). However, few observations on the suppressing activity of Aspergillus on M. furfur have been made.

In this study, the secondary metabolites of Aspergillus were profiled to classify Aspergillus strains into groups on the basis of cluster-specific properties by using LC–MS. Furthermore, several metabolites derived from Aspergillus were expected to inhibit M. furfur growth and the antimicrobial compounds were tentatively identified.

### Materials and methods

#### Chemicals and reagents

High performance liquid chromatography (HPLC)-grade water and methanol were purchased from Burdick and Jackson (Muskegon, MI, USA), and acetonitrile was purchased from Fisher Scientific (Pittsburgh, PA, USA). Malt extract broth, Czapek-Dox broth, and peptone were purchased from Becton Dickinson (Franklin Lakes, NJ, USA).

#### Fungal strains and culture conditions

Twenty-one Aspergillus strains (Table 1) were obtained from Korean Agricultural Culture Collection (KACC, Republic of Korea), Korean Culture Center of Microorganisms (KCCM, Republic of Korea), and Korean Collection for Type Cultures...
(KCTC, Republic of Korea). To evaluate the differences of secondary metabolites production from Aspergillus according to the culture medium and incubation time, Aspergillus was cultured on malt extract agar (MEA) and Czapek yeast extract agar (CYA) plates for 3 d, and young mycelia pieces (6 mm in diameter) were placed on fresh MEA and CYA plates and incubated at 28 °C (Larsen et al. 2007). After 3 d, three agar disks (6 mm diameter) and with mycelium were transferred to new MEA and CYA plates and incubated at 28 °C for 7, 12, and 16 d. The composition of each culture medium is as follows: CYA medium consists of 0.5% yeast extract (Becton Dickinson, Franklin Lakes, NJ, USA), 0.3% NaCO3, 3% sucrose, 0.13% K2HPO4, 9H2O, 0.05% MgSO4 7H2O, 0.05% KCl, 0.001% FeSO4 7H2O, 0.001% CuSO4 5H2O, 0.001% ZnSO4 7H2O, and 1.5% agar. And MEA medium is composed of 2% malt extract (Becton Dickinson, Franklin Lakes, NJ, USA), 2% glucose, 0.1% peptone (Becton Dickinson, Franklin Lakes, NJ, USA), and 1.5% agar. Malassezia furfur was cultured on modified Dixon’s agar (m Dixon’s) plates for 7 d at 36 °C. The composition of the Dixon’s medium was 3.6% malt extract, 0.6% peptone, 1.2% ox bile, 1.0% Tween 40, 0.2% glycerol, 2.0% oleic acid, and 1.5% agar (Miranda et al. 2007).

Extraction of fungal metabolites

The fungal disks (6 mm in diameter) grown on MEA and CYA for 7, 12, and 16 d were collected from four different plates (Smedsgaard 1997). The agar pieces were transferred to 3 mL of ethyl acetate and mixed on a rotary shaker at 200 rpm for 9 h in the dark, and the solvent was made to evaporate using a speed vacuum (Kang et al. 2011). The concentrated extracts were dissolved in 200 μL of HPLC-grade methanol, filtered through a 0.45-μm disposable polytetrafluoroethylene (PTFE) filter, and used for LC–MS analysis. Among Aspergillus spp., the ethyl acetate extracts of Aspergillus fumigatus 41200 showing the highest antifungal activity against Malassezia furfur KCTC 7743 were further purified by reverse-phase high performance liquid chromatography (RP-HPLC) by using an YMC Pack Pro C18 column (250 × 4.6 mm). The gradient elution of the mobile phase consisted of water with 5% acetonitrile (A) and 100% acetonitrile (B) was programmed as follows: 0–60 min, gradually increased from 5% B to 100% B; 60–70 min, 100% B; 70–80 min, sharply reduced to 5% B. The flow rate was 1 mL min⁻¹.

Liquid chromatography–electrospray ionisation tandem mass spectrometry (LC–ESI–MS–MS) analysis of Aspergillus metabolites

Liquid chromatographic analysis was performed on a Varian 500MS ion trap mass spectrometer (Varian, Inc., Palo Alto, CA, USA). The LC–MS system consisted of an LC binary gradient solvent delivery pump (Varian 212), a photodiode array detector (ProStar 335), and an auto sampler (ProStar 410). The LC system used in this study was a 100 × 2.0 mm C18 column with a 3-μm particle size (Varian). The MS was run in negative mode with the following parameters: drying temperature, 350 °C; needle voltage, 5 kV; capillary voltage, 70 V; drying gas pressure (nitrogen), 10 psi; nebuliser gas pressure (air), 35 psi. The running parameters were as follows: drying temperature, 350 °C; needle voltage, 5 kV; capillary voltage, 70 V; drying gas pressure (nitrogen), 10 psi; nebuliser gas pressure (air), 35 psi; and mass rage, m/z 100–1000. The binary mobile phase consisted of water and acetonitrile with 0.1% formic acid (v/v). The initial gradient of the mobile phase was 10% acetonitrile for 2 min, and it was gradually increased to 100% acetonitrile over 28 min. The mobile phase was maintained at 100% acetonitrile for 5 min, and then sharply reduced to 10% acetonitrile in 0.06 min and maintained for 5 min. Each sample (10 μL) was injected, and the flow rate was maintained at 0.2 mL min⁻¹. Tandem mass spectrometry was performed using scan-type turbo data-dependent scanning (DDS) under the same conditions.

Ultraperformance liquid chromatography–quadrupole-time-of-flight–mass spectrometry (UPLC–Q-TOF–MS) analysis

Waters Micromass Q-TOF Premier with UPLC Acquity system (Waters, Milford, MA, USA) was used equipped with UPLC mass spectrometry by using an Acquity UPLC BEH C18 column (1.7 μm, 100 × 2.1 mm, Waters, MA, USA). Solvent A (0.1% v/v formic acid in water) and B (0.1% v/v formic acid in acetonitrile) were combined in order to be eluted with the mobile phase. The column was first-eluted with 0% B for 3 min, and gradient-eluted to 30% B over 3 min. It was further increased to 40% B for 1 min and then gradually increased to 100% B for 8 min. For each sample, 5 μL was injected, and the flow rate was maintained at 0.3 mL min⁻¹. The m/z 100–1000 was designated with the negative and positive mode range of ESI. The operating parameter of the negative and positive mode for the sample analysis was as follows: ion source temperature, 200 °C; cone gas flow, 50 L h⁻¹; desolvation gas flow, 600 L h⁻¹; capillary voltage, 28 kV; and cone voltage, up to 35 V.

Data processing

LC–MS data were analysed using Varian MS Workstation 6.9 software. The LC–ESI–MS negative mode chromatogram raw data files were converted into network Common Data Form (netCDF) format using Vx Capture2.1 software (Adron Systems LLC, Laporte, MN, USA). After conversion, the netCDF files were automatically processed with the mass spectrometry datasets by using MetAlign software package (http://www.metalign.nl) (Lommen 2009). The MetAlign parameters were set according to the specific scaling requirements: a peak slope factor of 1.0, peak threshold factor of 1.5, peak threshold of 100, and average peak width at half height of 7.0, which corresponds to a retention time of 2–35 min and mass range of 100–1000 for LC–MS. UPLC–Q-TOF–MS data were acquired using Waters MassLynx 4.1 software.

Multivariate statistical analysis

SIMCA-P+ 12.0 (Umetrics, Umea, Sweden) was used to perform the multivariate statistical analysis. The Log₁₀-transformed MS data was used in the unit variance scaling. PCA was used to observe the distribution of the Aspergillus strains. Each group was confirmed to be separated by the first principal component (PC1) and the second principal component (PC2). The Aspergillus strains were assigned to groups according to the results of
homologous metabolite profiling that coincided with those of hierarchical clustering analysis (HCA). The PLS-DA separated the groups from HCA and was a variable that was determined on the basis of the variable importance of the projection (VIP > 1.5) value and p-value (p < 0.005). Statistica 7 (StatSoft, Inc., Tulsa, OK, USA) determined the p-value between different metabolite-based cluster groups. The assignment of secondary metabolites contributing to the observed variance was performed by information with molecular weight, retention time, ultraviolet (UV) spectra, and MS² fragmentation pattern analysis obtained from LC−MS and Q-TOF−MS as well as by the published literature, Dictionary of Natural Products (Copyright 2008, Taylor & Francis Group, Boca Raton, FL, USA), and Antibase 3.0 (CambridgeSoft Corporation, Cambridge, MA, USA). The software prediction was compared with the simulated characterization of fragmentation as the MS² fragmentation pattern of each metabolite.

Agar diffusion method for antifungal assays

An agar diffusion method was used to determine the clear zone diameter of Aspergillus extract against the tested Malassezia furfur. The inoculum (100 μL) was sprinkled and diffused uniformly on m Dixon’s medium. Whatman sterile filter paper discs (6 mm in diameter) were covered with Aspergillus extract dissolved in 100 % methanol. Negative and positive controls were prepared using the same solvent employed to dissolve the Aspergillus extract (100 % methanol). The paper discs were placed on the agar surface, and the plates were incubated for 1 d at 37 °C (Miranda et al. 2007; Dung et al. 2008).

Results

Growth medium- and time-dependent metabolites in chemotaxonomy of Aspergillus

The metabolites of three Aspergillus sections—Fumigati, Nigri, and Flavi (total 21 strains)—were analysed by LC−MS negative-ion mode, and their metabolite profiles based on PCA score plots were determined at 7, 12, and 16 d of incubation on MEA (Fig 1A) and CYA (Fig 1B). Further, a dendrogram was used to cluster the Aspergillus strains on the basis of secondary metabolite profiles.

Fig 2 shows time-dependent chemotaxonomic features of MEA cultures for 7, 12, and 16 d. Each of the Aspergillus sections, Fumigati, Nigri, and Flavi, was separated on the basis of three different incubation times. The LC−MS-based dendrogram for MEA cultures was mainly divided into two characteristic branches. The first branch included section Flavi (Aspergillus flavus, Aspergillus oryzae, and Aspergillus sojae), and the second branch consisted of section Fumigati and Nigri strains. In the first branch, A. sojae strains belonged to section Flavi and were mixed with A. flavus and A. oryzae at 7 and 12 d of incubation. However, the metabolite profiles of A. sojae were separated from those of A. flavus and A. oryzae after 16 d of incubation.

Fig 1 – PCA score plots derived from the LC−MS datasets of Aspergillus spp. The Aspergillus strains were cultured on MEA (A) and CYA (B) media for 7, 12, and 16 d. A. fumigatus (○, AFU), A. niger (■, ANI), A. flavus (▲, AFL), A. oryzae (▲, AOR), and A. sojae (▲, ASO).
Metabolite-based dendrograms of the secondary metabolites from *Aspergillus* strains grown on CYA were used to cluster the three sections of *Aspergillus* (Fig 3). Most strains from separate sections were divided into separate clusters, except *Aspergillus fumigatus* KACC 41186 (section *Fumigati*) and *Aspergillus niger* KCTC 6960 (section *Nigri*), which were mixed with section *Flavi* in the analysis of 7 d of culture. In section *Flavi*, *A. flavus*, *A. oryzae*, and *A. sojae* species were separated in 12 and 16 d of culture. Additionally, the section *Flavi* species *A. sojae* was separated from *A. flavus* and *A. oryzae* in 16 d of incubation in CYA medium.

**Chemotaxonomy of Aspergillus spp. on the basis of metabolite profiling**

Among the 21 strains, 18 strains of *Aspergillus* spp. cultivated in MEA media for 12 d were analysed by LC–MS. The data combined with PCA separated *Aspergillus* into *Aspergillus fumigatus*, and three species (*Aspergillus flavus*, *Aspergillus oryzae*, and *Aspergillus sojae*) from the *Flavi* section (data not shown); these divisions were clearly confirmed by the HCA (data not shown). The three-group clustering of *Aspergillus* spp. was shown as PCA score plots with both PC1 and PC2. *Aspergillus niger* and three species (*A. flavus*, *A. oryzae*, and *A. sojae*) of the *Flavi* section were distinguished by PC1 (19.7 %), and *A. fumigatus* was separated from other species by PC2 (17.5 %). A dendrogram that classified the three characteristic branches on the basis of LC–MS data under the negative-ion mode was drawn. The first branch included *A. niger* KCTC 6960, KACC 40280, and KCCM 32005 and 60143. The second branch consisted of *A. fumigatus* KACC 41138, 41191, 41196, and 41200. The third branch consisted of three species (*A. flavus*, *A. oryzae*, and *A. sojae*) of the *Flavi* section (*A. flavus* KCTC 6984 and 16682 and KACC 40232; *A. oryzae* KACC 40067, 40234, and 40242, and KCCM 11896; *A. sojae* KACC 40072, KACC 41867, and KCCM 60354).

**Analysis of the secondary metabolites that distinguish Aspergillus fumigatus and Flavi sections**

*A. fumigatus* and three species (*Aspergillus flavus*, *Aspergillus oryzae*, and *Aspergillus sojae*) of the section *Flavi* were minutely analysed by PLS-DA score plot to further elucidate the secondary metabolites that divide these species into two groups (Fig 4). The orthogonal component with $R^2 = 0.246$ and $Q^2 = 0.874$ was used to analyse the metabolite profiles of the *A. fumigatus* and three species (*A. flavus*, *A. oryzae*, and *A. sojae*) of the *Flavi* section. The metabolites identified by VIP value (>1.5) and p-value (<0.005) were determined as the potential variables that separated groups in the dendrogram. Among the variables that divided species into two groups, 12 metabolites (gliotoxin G, fumigatin oxide, fumigatin, pseurotin A or D, fumiquinazoline D, fumagillin, helvolic acid, 1,2-dihydrohelvolic acid, 5,8-dihydroxy-9,12-octadecadienoic acid (5,8-diHODE), and two unidentified metabolites) were tentatively identified (Table 2), and their structures are shown in Fig. 5. The secondary metabolite quantities were calculated from LC–MS peak intensities from *A. fumigatus* and three species (*A. flavus*, *A. oryzae*, and *A. sojae*) of the *Flavi* section (Table 3). Other metabolites except for three metabolites (diHODE and two unidentified metabolites) were only detected from *A. fumigatus*. 
Antifungal activity against Malassezia furfur

The extracts of Aspergillus spp. were tested to evaluate the antifungal activity against M. furfur. The clear zone diameter indicated the intensity of the Aspergillus antifungal activity, suppressing M. furfur growth (Table 1). Among the Aspergillus spp., Aspergillus fumigatus showed the greatest species-specific activity against M. furfur. So, in order to elucidate of components with antimicrobial activity from A. fumigatus, A. fumigatus 41200 showing the highest activity was separated by HPLC. The peaks represent the antifungal activity were eluted at retention times of 39, 42, and 43 min (data not shown). The m/z value of each peaks were 457, 567, and 569 by LC eESI-MS under negative-ion mode and high resolution UPLC eQ-TOF eMS indicated the m/z values of each compounds at [M – H] – 457.2223, 567.2947, and 569.3110. The candidate formulae were estimated as C26H33O7, C33H43O8, and C33H45O8 from the m/z values. Based on the above data, three compounds with antifungal activities from A. fumigatus were tentatively identified as fumagillin, helvolic acid, and 1,2-dihydrohelvolic acid, respectively. These active compounds also were major fungal metabolites that contributed to the discrimination between A. fumigatus and three species (Aspergillus flavus, Aspergillus oryzae, and Aspergillus sojae) of the Flavi section.

Discussion

In this study, different Aspergillus strains were separated according to their sections. The culture medium-dependent and time-dependent secondary metabolite profile-based chemotaxonomy results were analysed by LC–ESI-MS–MS. The tandem mass spectrometry method was used for metabolite identification because of their characteristic fragmentation patterns without the need for standard compounds. Also,
Table 2 — Identification of tentative compounds to be used as variables to classify *A. fumigatus* and three species of the Flavi section on the basis of LC–MS results.

<table>
<thead>
<tr>
<th>Tentative identification</th>
<th>( t_R ) (min)</th>
<th>M.W.</th>
<th>([M - H])</th>
<th>MS(^n) fragment ions (m/z)</th>
<th>p-Value ((t)-test)</th>
<th>Measured ([M - H]) (Da)</th>
<th>Error (mDa)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gliotoxin G (1)</td>
<td>8.9</td>
<td>390</td>
<td>( *391 &gt; 373, 364 )</td>
<td>1.30E–03</td>
<td>388.9750</td>
<td>–2.1</td>
<td>Frisvad et al. (2009)</td>
<td></td>
</tr>
<tr>
<td>Fumigatin oxide (2)</td>
<td>9.1</td>
<td>184</td>
<td>183 &gt; 167, 124, 139, 138</td>
<td>2.71E–03</td>
<td>183.0290</td>
<td>–2.2</td>
<td>Frisvad et al. (2009)</td>
<td></td>
</tr>
<tr>
<td>Fumigatin (3)</td>
<td>12.2</td>
<td>168</td>
<td>167 &gt; 151, 108</td>
<td>1.08E–04</td>
<td>167.0341</td>
<td>–1.8</td>
<td>Frisvad et al. (2009)</td>
<td></td>
</tr>
<tr>
<td>Pseudotrin A or D (4)</td>
<td>13.0</td>
<td>431</td>
<td>269 &gt; 157, 133</td>
<td>2.01E–03</td>
<td>265.9467</td>
<td>2.6</td>
<td>Maiya et al. (2007)</td>
<td></td>
</tr>
<tr>
<td>Pseudotrin A or D (5)</td>
<td>13.2</td>
<td>431</td>
<td>430 &gt; 334, 149</td>
<td>6.30E–05</td>
<td>454.1467</td>
<td>2.6</td>
<td>Maiya et al. (2007)</td>
<td></td>
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<tr>
<td>N.I. (6)</td>
<td>14.6</td>
<td>546</td>
<td>545 &gt; 417, 311</td>
<td>3.47E–05</td>
<td>545.2031</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>N.I. (7)</td>
<td>15.9</td>
<td>466</td>
<td>465 &gt; 402, 382</td>
<td>3.28E–04</td>
<td>456.1819</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Fumiquinazoline D (8)</td>
<td>16.1</td>
<td>443</td>
<td>442 &gt; 240, 212, 225</td>
<td>2.40E–03</td>
<td>424.1507</td>
<td>–1.7</td>
<td>Larsen et al. (2007)</td>
<td></td>
</tr>
<tr>
<td>diHODE (9)</td>
<td>19.2</td>
<td>312</td>
<td>311 &gt; 293, 173 &gt; 275, 155</td>
<td>6.04E–05</td>
<td>311.2219</td>
<td>–1.0</td>
<td>Mazur et al. (1991), Ishikawa et al. (2009)</td>
<td></td>
</tr>
<tr>
<td>Fumagillin (10)</td>
<td>22.2</td>
<td>458</td>
<td>481 &gt; 437, 399, 358, 305, 287, 123</td>
<td>7.69E–04</td>
<td>457.2223</td>
<td>–0.7</td>
<td>Frisvad et al. (2009)</td>
<td></td>
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<tr>
<td>Helvolic acid (11)</td>
<td>22.7</td>
<td>568</td>
<td>509 &gt; 403, 385, 343, 213</td>
<td>1.08E–08</td>
<td>567.2947</td>
<td>–1.7</td>
<td>Fox et al. (2004), Lee et al. (2008)</td>
<td></td>
</tr>
<tr>
<td>1,2-Dihydrohelvolic acid (12)</td>
<td>22.9</td>
<td>570</td>
<td>511 &gt; 405, 387, 345, 215</td>
<td>1.73E–05</td>
<td>569.3110</td>
<td>–0.7</td>
<td>Lee et al. (2008)</td>
<td></td>
</tr>
</tbody>
</table>

\( t_R \), retention time; M.W., molecular weight; Ref., reference; N.I., not identified; \( * \), positive mode.
\( a \) Significant difference by \( t \)-test.

Multivariate statistical analysis determined characteristic metabolites of *Aspergillus* sections. The culture medium-dependent and time-dependent secondary metabolite profile-based chemotaxonomies separated *Aspergillus* strains into section *Fumigati*, *Nigri*, and *Flavi*. In most of the *Aspergillus* strains, the changes of secondary metabolites production from *Aspergillus* had more similar chemotaxonomic features at 16 d of cultivation time than 7 and 12 d of incubation in both culture medium conditions. In section *Flavi*, *Aspergillus sojae* strains were clustered with *Aspergillus flavus* and *Aspergillus oryzae* grown in MEA for 16 d and in CYA for 12 and 16 d. An internal transcribed spacer-based phylogenetic tree also demonstrated that the cluster of *A. sojae* was different from those of *A. flavus* and *A. oryzae*.

![Fig 5](image_url) — Structures of significantly different metabolites among *A. fumigatus* and three species of the Flavi section.
Production of secondary metabolites of *C.* by detected using LC–ESI-MS–MS negative mode.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>M.W.</th>
<th>Isolation time (min)</th>
<th>Fumigati</th>
<th>A. nigri</th>
<th>A. sojae</th>
<th>A. oryzae</th>
<th>A. flavus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gliotoxin G</td>
<td>41138</td>
<td>8.9</td>
<td>+++</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>Fumigatin oxide</td>
<td>41191</td>
<td>8.9</td>
<td>+++</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>Fumigatin</td>
<td>41196</td>
<td>12.2</td>
<td>+++</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>Pseurotin A or D</td>
<td>41200</td>
<td>14.6</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>Gliotoxin D</td>
<td>6960</td>
<td>16.2</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>Fumigatin oxide</td>
<td>40230</td>
<td>19.2</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>+++</td>
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<td>+</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>+++</td>
</tr>
</tbody>
</table>

* R, retention time; M.W., molecular weight; N.I., not identified.

### Acknowledgements

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### References


