

***Aspergillus itaconicus* and *Aspergillus unguis* with New Addition to the Korean Flora**

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***Aspergillus itaconicus* 및 *Aspergillus unguis*를 추가한 韓國의 *Aspergillus***

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**Abstract**

Two hundred and twenty five strains which belong to the genus *Aspergillus* were isolated from specimens collected throughout the southern coastal areas of Korea. Fifteen species and one variety throughout the nine species groups including *A. itaconicus* and *A. unguis*, which are unrecorded in Korea, were identified according to Raper-Fennell's classification key.

**INTRODUCTION**

Since the year of 1940, taxonomical studies on the genus *Aspergillus* have advanced greatly. 17 species, 8 varieties and 4 mutations were arranged into groups which showed related physiological or biochemical activities in "A Manual of *Aspergilli*" by Thom and Raper in 1945. Scores of new species of *Aspergillus* have been described or newly discovered for several years since 1945. The need for enlargement of some groups and recognition of five new groups led Raper and Fennell to publish "The Genus *Aspergillus*" in 1965, which contained 132 species and 18 varieties throughout 18 species groups.

In Korea, 24 species and 1 variety included in 13 species groups were described by mycologists, Lee and Chang, Lee and Kim, Kim, Lee and Lee, *et. al.*, from 1964

to 1976. In addition to the previously described species, 2 strains were identified as *A. itaconicus* and *A. unguis* which have not been described before in Korea.

*A. itaconicus* was isolated originally from the juice of salted plums and described by Kinoshita in 1931. This species was at first arbitrarily placed at the end of the *A. restrictus* series of the *A. glaucus* group by Thom and Raper(1945) because of its osmophilic character and the form and dimension of its conidia; even though it differed markedly from other members of the series in many morphological details. In 1965, *A. itaconicus* placed in the newly established *A. cremeus* group with four new species of subtropical origin by Raper and Fennell.

*A. unguis*, isolated originally from soil, is characterized by sterile spicular hyphae that are absent in other species of the *A. nidulans*

group. Raper and Fennell regarded specular hyphae as singularly diagnostic for this species, although cleistothecia and ascospores produced in some strains were reported by them in 1955.

In the present study, we isolated 225 strains from various specimens and identified them according to the key of Raper-Fennell's.

## MATERIALS AND METHOD

### 1. Experimental materials

The experimental materials used in the present study were collected throughout the southern coastal areas of Korea from July to December in 1975. Substrates from which *Aspergilli* strains were isolated are soil, cereals, vegetables, fruits, bread, Korean rice cake, meju, bean paste, dried, fishes, beanpod, soybean sauce, etc. Among the 225 strains which were isolated from various substrates, strain M 4 and strain M 26 which were identified into *A. itaconicus* and *A. unguis*, were isolated from the substrates of meju and steamed rice, respectively.

### 2. Isolation and maintenance of strains

10-15 cc. of Pfeffer's solution were poured into vial containing samples and cultured for 5 to 10 days. Then conidia were inoculated on Czapek's agar medium.

After incubation at 24°-26°C for 10 days to 12 days, the strains of *Aspergillus* were isolated on the basis of their morphological characters. Further pure culture was obtained by two or more successive subcultures of a single colony from agar medium; and was maintained on the slant agar at 4°-5°C and subcultured every two months.

### 3. Identification of species

The isolated 225 strains of *Aspergillus* were incubated on Czapek's solution agar, malt extract agar, M 40 Y agar and Czapek's solution agar, containing 20 percent sucrose, respectively, at 24°-26°C for 12 days to 2 weeks. Colony morphology was observed in each strain and characters was investigated under the microscope, size and color of conidial heads, conidiophore, conidium, vesicle and sterigmata. Then they were examined whether or not of formation, shape, size and color of cleistothecium, ascospores, ascus, sclerotium and hüll cells according to Raper and Fennell's classification key.

## RESULT

Among the 225 strains of *Aspergilli*, 197 strains were identified into 9 species groups including 15 species and 1 variety according to Raper-Fennell's classification key. But the rest of them could not determine the name of species. Species descriptions were observed on the Czapek's solution agar, malt extract agar, M 40 Y agar and Czapek's solution agar containing 20 per cent sucrose at 24°-26°C for 12 days to two weeks, and is based upon the morphological or physiological aspects of 2 strains that are unrecorded in Korea.

The following is a list of Korean *Aspergillus* which were arranged into 26 species and 1 variety including *A. itaconicus* and *A. unguis*, which have been described to the present in Korea.

### 1. Description of species

*A. itaconicus*: Colonies on Czapek's solution agar grew fairly, at 24°-26°C attaining a diameter of 3 to 5cm. in 12 days to 2 weeks; forming dense felts 1 to 2mm. deep, white, ridged and radially

A list of Korean *Aspergillus*

| Species groups       | Species                                    | Nomenclator    | Describer                                  |
|----------------------|--|----------------|--|
| <i>A. clavatus</i>   | <i>A. giganteus</i>                        | Wehmer         | Lee, Kim(75)                               |
|                      | <i>A. clavatus</i>                         | Desmazieres    | Lee, Kim, Lee(68) Kim(71)                  |
| <i>A. glaucus</i>    | <i>A. pseudoglaucus</i>                    | Blochwitz      | Lee, Kim(75)                               |
|                      | <i>A. ruber</i>                            | Thom & Church  | Kim(71)                                    |
| <i>A. ornatus</i>    | <i>A. spinulose</i>                        | Warcup         | Lee, Kim(75)                               |
| <i>A. fumigatus</i>  | <i>A. fumigatus</i>                        | Fresenius      | Lee, Kim, Lee(68) Kim(71)<br>Lee, Kim(75)  |
| <i>A. ochraceus</i>  | <i>A. ochraceus</i>                        | Wilhelm        | Kim(71) Lee(75)                            |
|                      | <i>A. sulphureus</i>                       | Thom & Church  | Lee, Kim, Lee(68) Lee (75)                 |
| <i>A. niger</i>      | <i>A. ficuum</i>                           | Hennings       | Lee, Kim(75)                               |
|                      | <i>A. phoenicis</i>                        | Thom           | Lee, Kim, Lee(68) Lee(75)                  |
|                      | <i>A. niger</i>                            | Van Tieghem    | Lee, Kim, Lee(68) Kim(71)                  |
|                      | <i>A. tubingensis</i>                      | Mosseray       | Kim(72)                                    |
|                      | <i>A. pulverulentus</i>                    | Thom           | Kim(71)                                    |
|                      | <i>A. japonicus</i>                        | Saito          | Lee, Kim(75)                               |
| <i>A. candidus</i>   | <i>A. candidus</i>                         | Link           | Lee, Kim(75)                               |
| <i>A. flavus</i>     | <i>A. flavus</i>                           | Link           | Lee, Kim, Lee(68) Kim<br>(71) Lee, Kim(75) |
|                      | <i>A. flavus</i> var.<br><i>columnaris</i> | Link           | Lee, Kim(75)                               |
|                      | <i>A. oryzae</i>                           | Cohn           | Lee, Kim, Lee(68) Kim<br>(71)              |
| <i>A. wentii</i>     | <i>A. parasiticus</i>                      | Speare         | Lee, Kim, Lee(68)                          |
|                      | <i>A. thomii</i>                           | Smith          | Lee, Kim(75)                               |
| <i>A. versicolor</i> | <i>A. versicolor</i>                       | Tiraboschi     | Kim(71) Lee(75)                            |
|                      | <i>A. sydowi</i>                           | Thom & Church  | Kim(71) Lee(75)                            |
|                      | <i>A. silvaticus</i>                       | Raper, Fennell | Kim(72)                                    |
| <i>A. flavipes</i>   | <i>A. flavipes</i>                         | Thom & Church  | Lee, Lee(75)                               |
| <i>A. nidulans</i>   | <i>A. nidulans</i>                         | Wint           | Lee, Kim, Lee(68) Kim(71)<br>Lee, Kim(75)  |
|                      | <i>A. unguis</i>                           | Thom & Raper   | Lee, Kim, Kang(77)                         |
| <i>A. cremeus</i>    | <i>A. itaconicus</i>                       | Kinoshita      | Lee, Kim, Kang(76)                         |

furrowed; produced scattered long-stalked brownish shade conidial heads; exudate produced in yellowish droplets; reverse uncolored to yellowish shade. Both growth rate and sporulation were further en-

hanced upon Czapek's solution agar containing 20 per cent sucrose and measured 6 to 7cm. in diameter of colony in 2 weeks.

Colonies on M40Y agar grew more

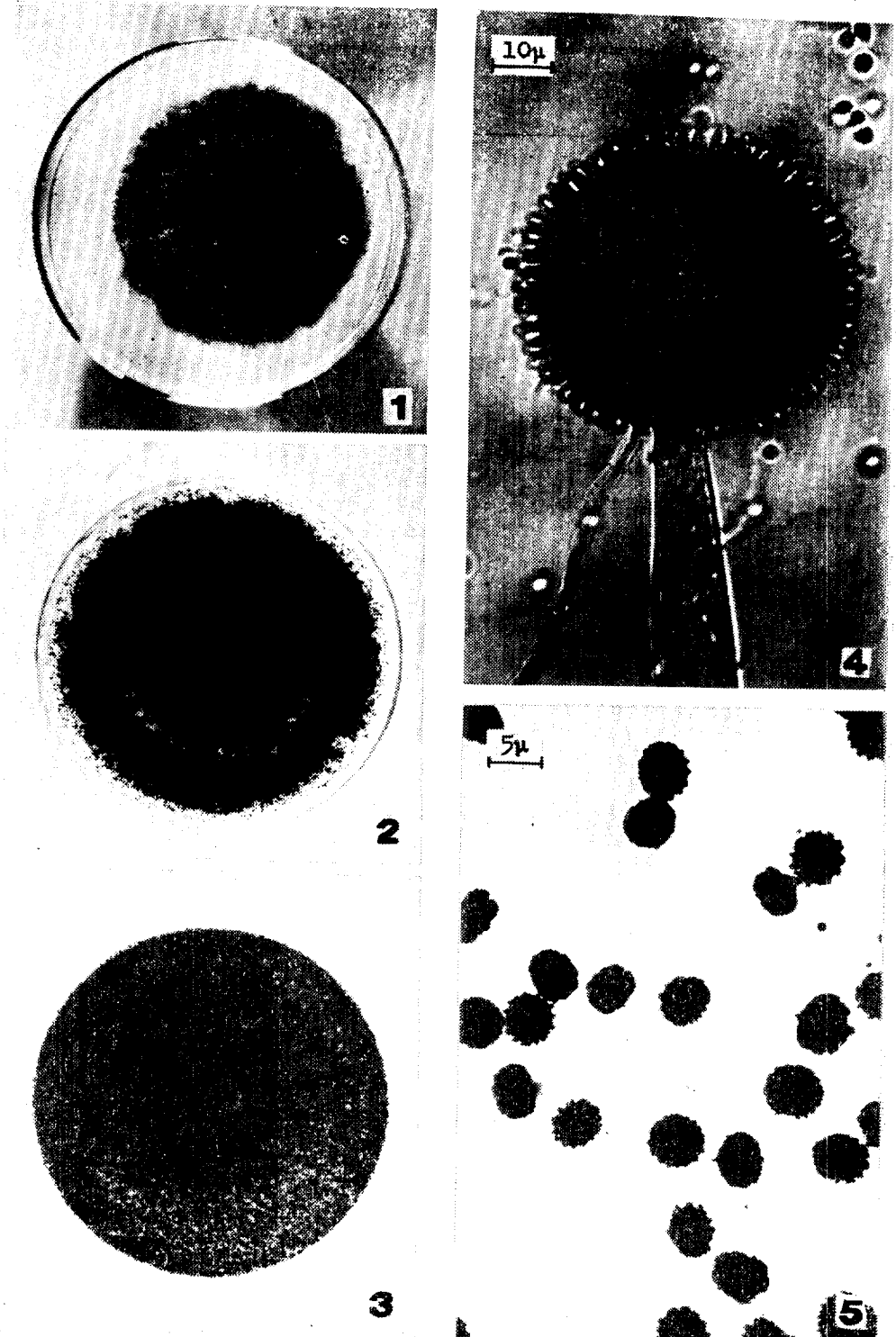


Fig. 1—5: *Aspergillus itaconicus*

Fig. 1. Colonies on Czapek's solution agar, 2 weeks at 24°—26°C

Fig. 2. Colonies on Czapek's solution agar containing 20 per cent sucrose

Fig. 3. Colonies on M 40 Y agar

Fig. 4. Conidial head      Fig. 5. Conidia

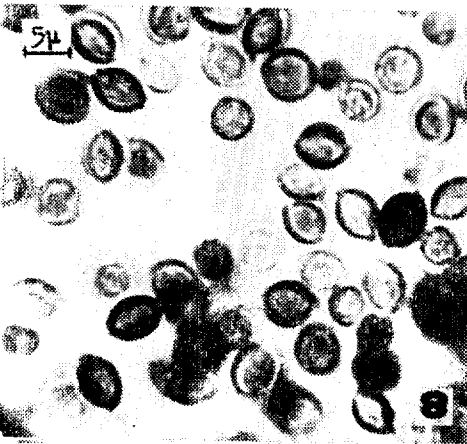
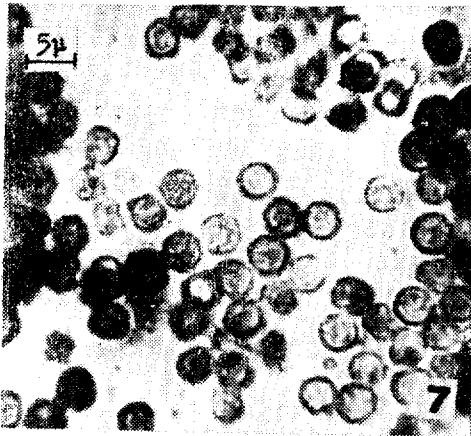
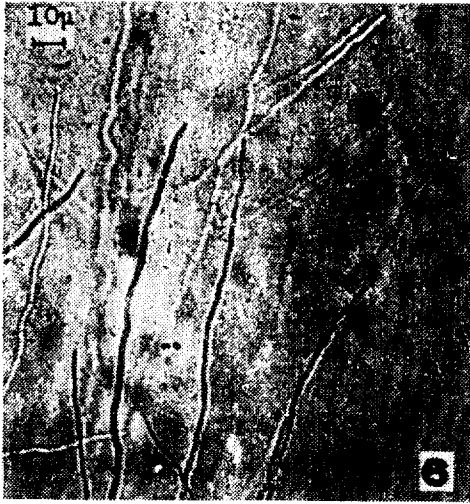


Fig. 6-10: *Aspergillus unguis*

Fig. 6. Sterile spicular hyphae showing typical of the species

Fig. 7. Conidia

Fig. 8. Ascospores on malt extract agar

Fig. 9. Conidial heads

Fig. 10. Globose hull cells on malt extract agar

rapidly at 24°—26°C, attaining a diameter of 8 to 10cm. in 2 weeks; consisted of a basal mycelium with white floccose aerial growth 2 to 3mm. deep covering the entire colony surface and continuing up the sides of the dish; conidial heads, pale brown in the young becoming brown with age, produced fairly abundant throughout but slightly more crowded in marginal areas; exudate lacking; reverse bright golden yellow. Conidial heads globose to radiate or radially split few in number, variable in size, most commonly 300 to 450 $\mu$ , up to 600 $\mu$  in diameter; conidiophore smooth, colorless, most commonly 1.5 to 2mm., up to about 3mm. in length, with walls 1.0 to 2.0 $\mu$  thick, conspicuously constricted just below the vesicles to diameters of 5.0 to 7.0 $\mu$  then increasing rather abruptly to diameters of 11 to 15 $\mu$ , which remain more or less constant throughout their entire length; sterigmata uniseriate, beared entirely over the vesicle, 7.0 to 11 $\mu$  by 2.0 to 3.0 $\mu$ ; conidia more or less pyriform to elliptical, echinulate, 4.0 to 5.0 $\mu$  by 3.0 to 4.0 $\mu$ ; vesicle globose, variable in size, most commonly 30 to 40 $\mu$ , up to 60 $\mu$  in diameter.

*A. unguis*: colonies on Czapek's solution agar grew more or less restrictedly at 24°—26°C, attaining a diameter of 2.5 to 3.5cm. in 12 days, plane, margins slightly lobed in yellowish green shade becoming brown with age; without cleistothecia or hüll cells; exudate lacking; reverse pale reddish. Microscopic examination revealed strikingly sterile spicular hyphae arising from foot cells or mycelial cells, with walls roughened and brownish shade; typically erect and slanting upward but sometimes sinuous, up to 1000 $\mu$  or more in diameter. Conidial heads coul-

nar, yellowish green at first becoming brown with age, 70 to 140 $\mu$  by 40 to 50 $\mu$ ; conidiophore smooth walled, dull brown, mostly 45 to 85 $\mu$  by 4 to 6 $\mu$ ; vesicle usually hemispherical or subglobose, measuring 9 to 12 $\mu$  in diameter, fertile over the upper three fourths; sterigmata in double series, primary sterigmata 5 to 6 $\mu$  by 2 to 2.5 $\mu$ , secondary sterigmata 5 to 6 $\mu$  by 1.5 to 2 $\mu$ ; conidia globose, rugulose, dull green, 3 to 3.5 $\mu$  in diameter.

Colonies on malt extract agar grew more rapidly, attaining a diameter of 3.5 to 4cm. in 12 days, plane, heavily sporulating entirely throughout, artemesia green, with submerged margins entire; exudate lacking; reverse uncolored. Conidial structure as described on Czapek's solution agar. Cleistothecia occurred on malt extract agar, globose to subglobose, usually 150 to 250 $\mu$  in diameter, surrounded by a thin envelope of globose hüll cells of 10 to 20 $\mu$  in diameter; asci observed as eight-spored, ovoid to subglobose, 9 to 12 $\mu$  in diameter; ascospores lenticular, purplish red with smooth convex walls and two equatorial crests, about 4 to 5.5 $\mu$  by 3 to 3.5 $\mu$ .

## DISCUSSION

*Aspergillus cremeus* group is divided into 2 subgroups by Raper and Fennell according to the coloration of their conidial heads. The isolated strain M 4 showed a marked resemblance to the *A. chrysellus* series in its brownish, large, loosely radiate conidial heads borne on long stalks and in its large, globose vesicles that are fertile over its entire surface. Its appearance, however, differed definitely from the *A. chrysellus* series in the morphological aspects of its colony and

sterigmata, and also in producing no cleistothecia or hüll cells. Enhancement of the growth rate and sporulation were observed upon the substrate containing 20 percent sucrose. Furthermore, its osmophilic character, which is diagnostic for this species group, were evidenced by its more preference for substrate which containing 40 percent sucrose, such as M 40 Y agar. It was regarded as a physiological relationship to the *A. glaucus* group. Dense basal mycelium with white floccose aerial growth, 2 to 3mm. deep, were also observed in the colonies on M 40 Y agar, which covered the entire colony surface and continued up the sides of the dish after 1 month.

Strain M 26, isolated from steamed rice, revealed striking sterile spicular hyphae that were regarded as the general pattern of those present in *A. unguis*. Cleistothecia and ascospores which were produced on malt extract agar may be considered as the same type of *A. unguis* as reported

by Raper and Fennell(1955). In the identification of strain M 26, we followed Raper and Fennell's consideration of which sterile spicular hyphae is a singularly diagnostic of *A. unguis* and regarded strain M 26 as *A. unguis* with ascospore stage; which is not believed as a representative of *A. unguis* with noncleistothecia and nonascospores.

An additional strain M 74, isolated from soil, which also produced cleistothecia and ascospores, showed spicular hyphae that were long and sinuous rather than erect or slanting. In spite of the fact that strain M 74 produced spicular hyphae, the name of the species was not determined as *A. unguis* because of its many different morphological characters which showed morphological and cultural similarities to *A. nidulans*. However, the presence of spicular hyphae, which are absent in *A. nidulans*, indicate a relationship to *A. unguis*.

## 摘 要

우리나라 중남부 해안지역에서 수집된 표본들로부터 분리된 *Aspergillus* 屬에 속하는 225군주를 순수배양하고 Raper-Fennell의 분류방법에 따라 동정한 결과 9종군에 걸쳐 15종 1변종을 확인할 수 있었는데 그들중 한국 미기록종은 다음과 같다. *Aspergillus itaconicus*, *Aspergillus unguis*.

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