ÖZET


SUMMARY

Three *Fusarium* species (*Fusarium equiseti, Fusarium graminearum* and *Fusarium moniliforme*) were preserved at 4°C in a sterile soil medium from 1986-1992. At the end of this time, colony characteristics and morphological properties are examined on various media.

INTRODUCTION

As we study *Fusarium* species as pure cultures, species must be stored in sterile media. Media are different and they are selected according to the purpose of study. Species can be transferred to different media in the tuve for a short-term preservation. However, media, such as potato Dextrose Agar (PDA) are unsuitable for a long-term preservation of *Fusarium* species. For example, a species of *Fusarium* can live for 5-8 months in the media; but this period may increase or reduce depending on the amount of medium, temperature of environment and water content of medium. Specimen must be transferred to new medium after this period. In the contrary case, specimen may die. Specimens must be transferred at once at six months. But, there are several problems in this procedure:

1. The time and effort that are consumed for this procedure.
2. Consumption of media and other expenditures.

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3. Losing of the specimen as a result of the probably contaminants that may occur during transfer.

4. Losing of morphological characteristics belonging to specimen, if doing transfer very often (1).

5. Increase of mutation frequency (2).

For these reasons, other methods, such as liquid nitrogen (2), soil tubes (2-4), silica gel (5, 6) and lyophilization (7, 8) have been used for preservation of *Fusarium* species. Especially lyophilization is common (7, 8). In addition, the preservation in the mineral oil is proposed (9). Usually, soil is suitable for living of microfungi, therefore reflection of preservation in the soil is attractioned to researchers. Booth (7) stated that the method of the preservation of species in the soil is a cheap method. However, he stated that it is not dependable and stressed that mutations may occur during preservation of *Fusarium* species in the soil tube. Superior points of soil tube may be summarized as follows (10):

1. The living time of specimens increases.

2. Carrying out of soil tube is easy and simple.

3. If soil tube method is applied well, it doesn’t destroy homojenicy of specimens.

But, we can’t observe the developmental characteristics and understand contamination (10). In addition, some morphological characteristics may be lost (6).

Miller and Cormack described preservation of *Fusarium* species in sterile, sand and clay soils (2). Cormack found *Fusarium avenaceum* (Fr.) Sacc. in the soil that can live 3 years at room temperature (2).

Stover and Frieberg (11) examined the effect of carbon dioxide as *Fusarium* species multiply in soil. These researchers used clay and sandy soils. McKeen and Wensley (3) studied the longevity of *Fusarium oxysporum* Schl. Emend, Snyd. & Hans. in soil tube cultures. These researchers preserved the specimens for 3 years in soil tube cultures and determined no morphological variation.

Griffin and Pass (12) examined the production state of macroconidium and chlamydospore of *Fusarium roseum* "Sambucinum" under carbon starvation conditions in relation to survival in soil. Qureshi and Page (13) also examined chlamydospore production state in two salt solutions. Klotz et al. (14) examined the production state of chlamydospore too. Windels et al. (5) stored different *Fusarium* species in silica gel and soil during 5 years in 4.5°C. These researchers stated that 95 %, 93 % and 94 % of 461 isolates remained alive in the soil on 3rd, 4th and 5th years, respectively. Bacon et al. (15) described medium for isolation of peritecial state of *Fusarium graminearum* Schwabe. This medium included soil extract-corn meal agar.
MATERIALS AND METHODS

A total of 17 isolates belonging to 3 *Fusarium* species (*Fusarium moniliforme* Sheldon (6 isolates), *F. graminearum* (5 isolates) and *Fusarium equiseti* (Corda) Sacc. (5 isolates)) are preserved on sterile soil cultures from 1986-1992. Specimens were isolated from corn seeds (16).

Sterile soil media are prepared as below (This formula is obtained from Institute of Mycology in Berlin, Germany):
- 2 part normal soil.
- 2 part compost soil.
- 1 part sand (Dried by air and had been to sift).

500 grams of this mixture were mixed with 20 ml tapwater. This mixture was put into tubes to a height of 6-7 cm and 1.5 cm tapwater was added and they were let wait for 24 hours. Then the tubes were sterilised in autoclave under 2 atmosphere pressure for 60 minutes. This procedure was done 3 times at 24 hour-intervals.

Specimens were inoculated to these soil media and stored at 4°C. 3-3.5 ml sterile-pure water was added to each tube at one year intervals.

Specimens were inoculated to PDA and Czapek Dox Agar (CDA) (Oxoid) media at the end of the six years and probable changes were examined.

RESULTS AND DISCUSSION

A total of 17 isolates belonging to three *Fusarium* species are preserved on sterile soil cultures for six years. At the end of six years, all the above-mentioned were alive. When inoculated to PDA and CDA media, specimens grew in these media. Important differences from the point of colony were not observed. McKeen and Wensley (3) also found similar results. But, some differences were observed in macroconidia of *F. equiseti*. The tip parts of macroconidium were blunted, septa were hardly seen and structures similar to chlamydospores occured on macroconidia of *F. equiseti*. In addition, the width of macroconidia increased less, while the mean width of macroconidia with 3 and 4 septa were 3.96 microns six years before (The mean value of 100 macroconidia were calculated); they were found as 4.74 microns of mean value of macroconidia with 4 septa after six years (The mean value of 30 macroconidia were calculated). No morphological variation was observed in chlamydospores. Morphological differences and coloni colours in other two species were also not seen. Some colony characteristics of three *Fusarium* species in CDA and PDA are stated below:

*F. graminearum* (In CDA, 3 weekly coloni): There are aerial mycelium, cottonish, white, rose [CIC 17]: 39, yellowish (CIC: 49), underside is red (CIC: 42) to yellow (CIC: 9H) and cream (CIC: 5E).

In PDA, 4 daily coloni: Growth is rapid, 5.5 cm/4 days. There are aerial mycelium, white, but after two days reddish, especially in center. 4 th day the sides of
coli is white (CIC: 2B), cottonish, rose pink (CIC: 39) in center. Undersurface is blood red (CIC: 41) the sides of coloni are buff (CIC: 52).

_F. equiseti_ (In CDA, 3 weekly coloni): There are aerial mycelium, cottonish, white (CIC: 1A), brownish to cinnamon (CIC: 10), cream (CIC: 4D) on center, undersurface is yellow (CIC: 6F) to deep yellow (CIC: 9H).

In PDA, 4 daily coloni: Growth is rapid, 5.2 cm/4 days, there are aerial mycelium, cottonish, white, undersurface is cream to buff (CIC:52).

_F. moniliforme_ (In CDA, 3 weekly coloni): There are aerial mycelium, cottonish, white, hardly visible is pinkish, undersurface is yellowish (CIC: 86) to buff (CIC: 52).

In PDA, 4 daily coloni: Growth is slow, 3-3.5 cm/4 days, there are limited aerial mycelium, cottonish, white to light purple on center (CIC: 79), undersurface is cream to buff (CIC: 52).

The sizes of conidia belonging to the Three _Fusarium_ species are given in Table 1, 2 and 3.

Table 1. The macroconidia sizes of _F. graminearum_ (Micron)

<table>
<thead>
<tr>
<th>The number of septa</th>
<th>Length</th>
<th>Width</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>34.88</td>
<td>5.66</td>
</tr>
<tr>
<td>5</td>
<td>40.29</td>
<td>5.99</td>
</tr>
</tbody>
</table>

Table 2. The microconidia sizes of _F. moniliforme_ (Micron)

<table>
<thead>
<tr>
<th>The number of septa</th>
<th>Length</th>
<th>Width</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>4.53</td>
<td>1.47</td>
</tr>
<tr>
<td>1</td>
<td>8.40</td>
<td>1.80</td>
</tr>
</tbody>
</table>

The _Fusarium_ species that were isolated from various substrates must be preserved. In this study, 17 specimens of _Fusarium_ genus were preserved in sterile soil tube cultures for 6 years and were still alive after inoculation to PDA and CDA, and there was no observed contamination.

There are various causes of becoming alive of specimens in soil cultures. Soil tubes provide advantage, for these fungi are similar to natural environment. Because specimens are stored in 4°C; metabolism slows down. 3-3.5 ml sterile-pure water was
Table 3. The macroconidia sizes of *F. equiseti* (micron)

<table>
<thead>
<tr>
<th>The number of septa</th>
<th>Length</th>
<th>Width</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>27.37</td>
<td>3.80</td>
</tr>
<tr>
<td>4</td>
<td>33.12</td>
<td>4.74</td>
</tr>
</tbody>
</table>

added to each tube at one year intervals. After six years, growth on PDA and CDA media of specimens might have been caused by chlamydospores. But *F. moniliforme* does not produce chlamydospore; therefore, multiplication occurs not only by chlamydospores; but by conidia and hyphae as well. However, the production of chlamydospore is important for specimens to survive for a long time. Toussoun and Nelson (4) proposed that a small piece of fungus must be put into sterile distilled water for the production of chlamydospores. Qureshi and Page (13) examined the production state of chlamydospores of *F. oxysporum* in some solutions. Researchers determined that organic and inorganic carbon source stimulate chlamydospore formation.

Although, there are some disadvantages of soil tube cultures, they also have advantages for preserving *Fusarium* species for use for 5-7, even 10 years (6). However, the probability of losing some morphological characteristics as a result of mutation is an important disadvantage. In addition, sometimes pathogenicity may lose (4, 6). Therefore, when preserving *Fusarium* species, the appropriate medium must be chosen. Windels et al. (6) proposed silica gel for the preservation of *Fusarium* species. According to these workers, silica gel is easy, mutation frequency is the least and cultures can repeatedly be taken out from a single storage tube. In addition, since contamination can not be understood in soil tube cultures, inoculation must be performed to a PDA medium at certain intervals and contamination must be controlled.

As a result, we can say that: When *Fusarium* species are preserved for a long-term, these must be made into consideration and appropriate medium must be chosen. But, although there are disadvantages in soil tube cultures, we can suggest to use soil cultures for preserving of *Fusarium* species.

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**REFERENCES**