Microbiological Studies on a New Species of *Oospora*,  
*Oospora astringenes* nov. sp. 

Part I. Identification and Mycological Properties 

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Investigation was made on the mycological properties of a species of *Oospora* which was isolated from the air of bronchial asthma patient’s room, Tokyo, Japan. 

This species was considered to be a new one and named as *Oospora astringenes* nov. sp.

On the malt extract glucose agar at room temperature, the colonies appear velvety, powdery and white. Conidia are ellipsoidal or cylindrical, mostly 7.0×4.3 µ in size, hyaline.

This new species utilizes only organic compounds as a sole source of nitrogen and its growth is markedly stimulated by the addition of some vitamins.

**INTRODUCTION**

The genus *Oospora* is well known as the plant-inhabitant organism, and it becomes sometimes the causative agent of plant diseases. For example, *Oospora citri-aurantii* is often found in citrus fruits and *Oospora pustulans* and *Oospora nicotiana* are causative agents of the skin spot of potato tubers and tabacco plant disease, respectively.

J. C. Gilman1) described five species from soil. T. Takahashi and coworkers2) reported that the members of *Oospora* were found most frequently in air-born fungi.

In the field of biochemistry, H. Nishikawa3) isolated sulochrin, spirane derivative and osoic acid from the mycelium of *Oospora sulphrea-ochracea*. *Oospora colorans* was found to produce oosporein by F. Kögl and G. C. van Wessem4). Y. Kodaira5) described destruxin A and B which were isolated from the culture media of *Oospora destructor* and toxic to some insects.

The authors6–8) isolated a species of the genus *Oospora* from the air of a patient’s room and described it’s metabolites, that is, oospolactone, C_{11}H_{6}O_{5} compound (O-2) and eburicoic acid.

In the present paper, the author describes mycological properties of the present species which has been named as *Oospora astringenes* nov. sp.

**METHODS**

Isolation of *Oospora astringenes* nov. sp.

Sabouraud’s and Waksman’s agar plates (9 cm diam. petri-dishes) were placed for ten minutes in the bronchial asthma patient’s room, Tokyo, Japan, July 1959. After a week at room temperature, a white

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3) H. Nishikawa, *This Journal*, 12, 47 (1936); 13, 1 (1937); 18, 13 (1942).
filamentous fungus was isolated from Sabouraud's agar plates on which various microorganisms developed. This fungus also grew on Waksman's agar. When the author examined the same place again in July 1960, this fungus was not found.

**Morphological Characteristics.**

The slide-culture method using malt extract glucose agar was employed, and the morphological study was carried out after culture for a week at 25°C.

**Culture on the Various Liquid Media.**

The conidia of seven days culture were inoculated into various liquid media in Fernbach flasks, each containing 200 ml of the medium (2 cm in depth) and cultured at 25°C under stationary condition.

Czapek-Dox's medium without sodium nitrate was used to examine the nitrogen requirement for it's growth.

Growth cycle of this species was examined by using the following medium.

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>20.0 g</td>
</tr>
<tr>
<td>Vitamin-free casamino acid</td>
<td>5.0</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>5.0</td>
</tr>
<tr>
<td>KH₂PO₄</td>
<td>1.0</td>
</tr>
<tr>
<td>MgSO₄·7H₂O</td>
<td>0.5</td>
</tr>
<tr>
<td>KCl</td>
<td>0.5</td>
</tr>
<tr>
<td>NaCl</td>
<td>0.1</td>
</tr>
<tr>
<td>CaCl₂·2HO</td>
<td>0.1</td>
</tr>
<tr>
<td>Dist. water</td>
<td>1000 ml</td>
</tr>
</tbody>
</table>

Growth rate was examined by weighing the cultivated mycelia after drying for 24 hours at 60°C.

The amount of glucose in the medium was determined by Somogy's method.

The pH of medium was determined with a glass electrode pH meter.

**RESULTS AND DISCUSSION**

**Mycological Properties and Description.**

*Oospora astringenes* nov. sp.

Hyphae well developed, septate, branched, 3.5~8.5 μ width, creeping, arising and intertwined on the agar media. Conidial hyphae develop from creeping or aerial hyphae and break up into conidia. Conidia are in a chain which branches and reaches to 200~350 μ in length, variously shaped, mostly long ellipsoidal or cylindrical, rounded at both ends, 4.0~11.5×3.0~5.5 μ, mostly 7.0×4.5 μ, hyaline (Fig. 1).

Growth on malt extract glucose agar, velvety, spreading on the whole surface of the agar. White at first, then becoming cream-white; reverse is pale yellow when matured. Knob-like hyphae develop arising and intertwined at the central area of the colony.

On potato-infusion agar, the colony is thin and powdery; reverse is pale yellowish color.

Colony on Czapek's agar, reaches to 5 cm in diameter in two weeks and submerged hyphae develop in the agar medium. It is hard to distinguish these submerged mycelia as they are poor-growing and hyaline.

On Sabouraud's medium, oat meal and broth agar as well as the medium just described, no formation of reproductive organs except for conidia could be observed even after prolonged cultivation.

The surface growth on the malt extract glucose medium is luxuriant, but the organism does not grow on Czapek-Dox's medium. On the surface culture of malt extract glucose medium, many islets of white colonies appear at first, then they spread on the whole surface. The medium colors yellowish after two weeks at 25°C, brownish after three weeks. The
Microbiological Studies on a New Species of *Oospora, Oospora astringenes* nov. sp.

The color of colony surface is lighter than that of medium. The culture media give aromatic and some irritative odour and astringent taste after about ten days.

**Hab.**; isolated from the air of patient room of bronchial asthma, Tokyo, Japan, July 1959. The type culture is preserved in the Institute for Fermentation, Osaka, Japan, in the following description.

*Oospora astringenes* nov. sp.

*Cultura in agarico maltato, velutina, puluracea, prino alba, deinde pallide cremea, reverso pallide lutescentes. Hyphae aeriales prostratae ramosae, fasciculatae septatae 3.5~8.5 μ, crassae. Conidia ellipsoidae vel cylindrica, 4.0~11.5×3.0~5.5 μ, hyalino."

Fungi, of which exogenous conidia are formed by dissociation of their hyphae, are classified into the genera *Oospora, Oidium* or *Geotrichum*, but the available descriptions of these genera are not sufficient enough to determine their systematic relationships.

For instance, D. H. Linder⁹) merged the genus *Oidium* in the dematiaceous fungi-imperfecti which include genus *Rhinotricum*. *Geotrichum* usually includes these of yeast-like, mucilaginous fungi. It is, therefore, reasonable that present species should be classified into the genus *Oospora*. In addition, the present species differs from those of *Coremilla* and *Coprotricium* in the mechanism of the spore formation.

The form-genus *Oospora* was divided into four groups by P. A. Saccardo¹⁰) from the color of conidia. G. Lindau¹¹) divided this genus into six groups, and C. Gilman classified five soil-inhabiting species into two groups by color of colonies.

Colonies of the present fungus are white and conidia are hyaline. Therefore, this fungus can be included in the first group of the above mentioned systems.

In Table I, the author selected nine species of fungi which are closely related to the present fungus. The present fungus is closest to *Oospora flocosa*, but differs from the latter in both form and size of conidia. Other species also differ from this fungus in the same items. Both *Oospora multiformis* and *O. gummigena* also resemble this fungus, but there are no descriptions available on conidial size of these fungi.

Accordingly, this fungus is considered to be an undescribed species of *Oospora* and the author gives a name as *Oospora astringenes* nov. sp. The epithet of this name is

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¹⁰) P.A. Saccardo, "*Sylloge Fungorum Omnium Hucusque Cognitorum*", 4, 11; 10, 512; 11, 583; 14, 1037; 16, 1024; 22, 1280; 25, 677 (1882~1931).
¹¹) G. Lindau, "Rabenhort’s *Kryptogamen-flora von Deutschland, Oestrreich und ver Schweiz.*" VIII, 25 (1907); IX, 718 (1910).
due to the astringent taste of the cultivated malt extract medium. This astringent taste is attributed to the metabolite, $O_2$ compound $C_{11}H_8O_5$, in the cultivated medium.

**The Growth Cycle of the Species.**

The growth curve of this fungus was investigated by weighing the mycelium of the liquid-culture, and at the same time the glucose consumption and pH-variation of culture medium were determined. Fig. 4 shows that seven to ten days may elapse before this fungus begins to grow rapidly and maximum growth is reached in three weeks. The glucose concentration in culture medium decreases as the growth proceeds, that is to say, the consumption of glucose is not found in the first week, but after ten days glucose is rapidly consumed and only a quarter of the initial dose remains after two weeks culture. Glucose is not found in three-weeks culture. Culture medium becomes acid after four days and the pH stays below 4.0 for three weeks, after then the medium turns alkaline.

When the present fungus is cultured on various liquid media (Fig. 5) containing such

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**Table I. Properties of Several Species Similar to Oospora astringenes nov. sp.**

<table>
<thead>
<tr>
<th>Species</th>
<th>Colonies</th>
<th>Mycelium</th>
<th>Conidia</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>O. verticilliods</em></td>
<td>Thin, white flat</td>
<td>Creeping, $70 \times 3 \mu$</td>
<td>Long spindle</td>
</tr>
<tr>
<td><em>O. multiformis</em></td>
<td>Lump, white powdery</td>
<td>Arising, branched</td>
<td>Various length, cylindrical</td>
</tr>
<tr>
<td><em>O. gummigena</em></td>
<td>White</td>
<td>Creeping, much branched and intertwined</td>
<td>Various form</td>
</tr>
<tr>
<td><em>O. pulmonoea</em></td>
<td></td>
<td>Thread-shaped, branched $5 \sim 10 \mu$ diam.</td>
<td>Elliptical or subglobose $5 \sim 10 \mu$ diam.</td>
</tr>
<tr>
<td><em>O. betae</em></td>
<td></td>
<td>Much branched $2 \sim 3 \mu$ diam.</td>
<td>Cylindrical or subglobose $14 \sim 16 \times 4 \sim 4.5 \mu$</td>
</tr>
<tr>
<td><em>O. gemmata</em></td>
<td></td>
<td>Creeping, $5.5 \mu$ diam.</td>
<td>Long cylindrical</td>
</tr>
<tr>
<td><em>O. citri-aurantii</em></td>
<td>White, cotton-like</td>
<td>Creeping, much branched $7.0 \sim 7.5 \mu$.</td>
<td>Cylindrical, $14.5 \times 7 \mu$</td>
</tr>
<tr>
<td><em>O. saccharina</em></td>
<td>White powdery</td>
<td>Creeping, thread-shaped $3 \sim 5 \mu$.</td>
<td>Egg-shaped, $12 \sim 13 \times 9 \sim 9.5 \mu$</td>
</tr>
<tr>
<td><em>O. floccosa</em></td>
<td>White cotton-like, wooly, cobwebby</td>
<td>Slender, intertwined branched $3.5 \sim 5 \mu$.</td>
<td>Spherical, $8 \sim 9 \times 5 \mu$</td>
</tr>
<tr>
<td><em>O. astringenes</em> (present species)</td>
<td>White velvety and powdery</td>
<td>Creeping or arising, much branched and Intertwined</td>
<td>Egg-shaped, $9.5 \sim 13 \times 5 \sim 8 \mu$</td>
</tr>
</tbody>
</table>

**Fig. 4. Consumption of Glucose and pH Change of Medium in Relations to Growth of Oospora astringenes nov. sp.**

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- **Weight of mycelium**
- **Glucose concentration g/l**
- **pH**

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FIG. 5. Growth of *Oospora astringenes* nov. sp. in Various Media.

I; Grape-Dox’s, II; Henneberg’s (peptone), III; Henneberg’s (potassium nitrate), IV; Mayer’s, V; Rusalin’s, VI; Sabouraud’s, VII, Malt ext. glucose.

organic nitrogen sources as peptone, casein and casamino acid, the good growth is obtained. On the contrary, it does not develop in the medium containing potassium nitrate, ammonium sulphate or urea as nitrogenous substrate.

Moreover, the growth of this fungus is stimulated by addition of malt and yeast extracts to the medium (Fig. 6). Therefore, this fungus utilizes only organic compounds

as a sole source of nitrogen and is considered to require some vitamins for the growth. On this problem, the author will report in the next paper.

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