Abstract

From the Antarctic materials, the following fungi were isolated. *Aspergillus repens*, *Asp. restrictus*, *Penicillium adametzi*, *Pen. canescens*, *Pen. charlesii*, *Pen. corylophilum*, *Pen. crustosum*, *Botryotrichum piluliferum*, *Chrysosporium pannorum*, *Ch. verrucosum*, *Dendryphiella salina*, *Monodictys austrina* and *Mucor mucedo*. Among them, *Dendryphiella salina* was isolated from many samples and its relationship to the marine environments was discussed; a new species, *Monodictys austrina*, was also described.

INTRODUCTION

The work reported here is a continuation and expansion of the previous work (1961) on the fungi isolated from the antarctic materials.

The antarctic continent and the regions surrounding it are the coldest area on the earth. Various types of microorganisms are maintaining their lives and reproducing under the rigorous environmental conditions. However, as to the kind of fungi living there, we have as yet very little information on the antarctic fungi even after the previous report was made (TUBAKI, 1961).

In 1962, MEYER, MORROW and WYSS found *Absidia corymbifera* and *Rhizopus arrhizus* from a food cache left by Captain R. F. SCOTT fifty years before at Cape Evans, Ross Island. They reported, in 1963, that none of the molds were discovered on any of the agar plates on which serial dilutions of frozen human feces and foodstuffs remained fifty years before were streaked. In the same year (1963), BOYD and BOYD reported on the soil microorganisms of Ross Island and the adjacent mainland. Their report was also concerned with the presence of *Penicillium* and *Aspergillus* species in the soil from the McMurdo Sound area.

Since the previous work was published, additional studies of the microflora of fungi have been carried out by the present authors and emphasis has been placed on the attempt to classify those fungi isolated from the antarctic materials and also to clarify their activity which can contribute to the ecological study of these regions. In the same cases, attention was paid to the ecological relationships between some antarctic fungi and the marine environments.

MATERIALS AND METHODS

Antarctic soil materials were collected mostly by Dr. H. FUKUSHIMA, Yokohama Municipal University, into the sterilized polyethylene sacks at McMurdo Sound and Cape Evans in 1962. From these materials, the fungi were isolated in the present laboratory immediately after their arrival at Japan. In the Antarctic, the direct-inoculation method (TUBAKI, 1961) of the soil onto malt agar slants (pH 4.0) was also employed by Fukushima, and the growing fungi were re-isolated at the present institute or at the Nagao Institute. In addition to the above fungi, unnamed cultures and the horse-dung were offered by Dr. Y. KOBAYASI, National

Science Museum, who spent several days of the brief austral summer (December-January, 1963) at the McMurdo Sound area and isolated a fungus from the mosseous material at the Bio-laboratory of the station.

Cultures, not described in the previous paper (1961), have also been studied and added to the present paper.

RESULTS

Identified species of fungi are listed in Table 1.

Table 1. Identified species of fungi.

Location / Species \ Date	Mc-Murdo	Cape Evans	Cape Royds	West Ongul	East Ongul	Syowa Station	Kind of Sample
	1962–1963			1962			
Asp. repens	1						soils
Asp. restrictus		1					soil
Botryotrichum piluliferum			1				horse dung
Chrysosporium pannorum	3	1					soil
Ch. verrucosum	2	1					soil
Dendryphiella salina	2	1		2	1	2	soil,moss,algae
Monodictys austrina	2						soil, algae
Mucor mucedo	1						soil
Penicillium adametzi	1						soil
Pen. canescens					1		soil
Pen. charlesii	1	1		1		1	soil
Pen. corylophilum		1			1		soil
Pen. crustosum	2	1					soil

SPECIES

Aspergillus repens (Cda.) De Bary (Plate 2-1)

Thom & Raper, A Manual of the Aspergilli, p. 103, 1945

One strain (NA 66-1) was isolated from the soil of McMurdo Sound (1962).

Aspergillus restrictus G. Smith

Thom & Raper, A Manual of the Aspergilli, p. 140, 1945

One strain (NA 71-1) was isolated from the soil of Cape Evans (1962). This species is characteristic in its restricted, dark green colony and is not uncommon on textiles. Our strain agrees well with the original description.

Botryotrichum piluliferum SACC. et MARCH. (Plates 1-C, 2-E, F)

Bull. Soc. Roy. Bot. Belg. **24**: 66, 1885
DOWNING, in Mycologia **45**: 934, 1953

Growth on malt agar rather restricted. Colony composed of white mycelium at first, then becoming brownish gray and powdery with conidia in abundance. Reverse yellowish to brownish. On potato agar, pale brown with pale olive-gray mycelium; reverse and agar yellow-orange with greenish tint. Conidiophores not well differentiated from the hyphae, branched in racemose in upper parts, hyaline. Conidia, the aleuriospore-type, acrogenous singly on each short-branch of the conidiophores, globose, double-walled, surrounded by a hyaline membrane, smooth, 10-20 μ, commonly 11-16 μ in diam., pale brown in mass. Sterile hairs borne individually or in tufts on the hyphae, smooth or rough, olive-gray to brownish, becoming paler upward, usually simple, 150-250×2.5-3.5 (4.0) μ, widening at the base to 5.0 μ.

Hab. On the horse-dung, collected by Dr. Y. KOBAYASI near SHACKLETON Hut, Cape Royds, Ross Island, Antarctica, Dec., 1963 (NA-BH).

Phialospore-production can not be found in the present strain and the sterile hair developed in culture differing from the DOWNING's description.

This species was originally found on dung in Belgium (1885), then on sacking in England (1919), on cheese in France (1924), and in soil in Canada (1953). In 1951, DOWNING studied *Botryotrichum* and *Coccospora* comparatively and reduced *Coccospora agricola* G. to synonymy under the present species. *C. agricola* was discovered in soil in Michigan (1913), then found on tentage in Hawaii (1914), and in peat in Michigan (1945).

The horse-dung, treated here, is considered to have been excreted by the horse taken by Ernest SHACKLETON of the British Expedition near the base camp in Cape Royds, Ross Island, in 1907-1909. Since then, the dung had remained frozen in snow and undisturbed for over 54 years until collected by Dr. Y. KOBAYASI in 1963. The dung retained the original shape when collected in the sterilized polyethylene sack, and it was divided into the two vertically and one half was put on the moistened sterilized filter paper in petri-dish and kept at 20°C at the present institute. After one week, whitish mycelia appeared on the whole surface of the dung, even on the central portion, and then, pale brownish conidia developed in abundance. These conidia were picked up by a sterilized needle onto the agar plates.

Chrysosporium pannorum (LINK) HUGHES

Canad. Jour. Bot. **36**: 749, 1958; CARMICHAEL, J. W., in Canad. Jour. Bot. **40**: 1137, 1962; TUBAKI, K., in Biol. Result. Jap. Antarct. Res. Exped., Seto Marine Biol. Lab. **14**: 6, 1961

This species was already found from the soil of West Ongul Island (TUBAKI, 1961) and characterized by the features of the colonial color, verticillate conidiophores and the small pear-shaped conidia (aleuriospores), 2-5×2-3.5(4) μ. In the present study, four strains of this species (NA 12-1, NA 64-1, NA 66-2, NA 66-b) were also isolated from the soil of McMurdo Station (1961) and Cape Evans

(1962). After CARMICHAEL (1962), the surface color of this species varies greatly and the same strain may show different colors on successive transfers, or even in different parts of one colony. However, the microscopic morphology is quite constant and characteristic as described already. Eventually, among the strains isolated, only one of them is typical *C. pannorum* and the other three strains differ from the typical one in rather gray-greenish colony, but their microscopic morphology agrees with that of the typical one.

Chrysosporium verrucosum TUBAKI

Biol. Result. Jap. Antarct. Res. Exped., Seto Marine Biol. Lab., **14**: 6, 1961

This species was originally isolated from the soil of West Ongul Island. It is characterized by the pale grayish green color of the colony with pinkish tint, dark brown colored reverse and the verrucose conidia, 3-5×2.8-3.0 μ. Through personal communication with Dr. Alfredo CORTE of the Institute Antarctica Argentino, the author learned that this species was also found in Decepcion Island. In the present study, three strains (NA 63-1, NA 65-1, NA 67-1) were isolated from the soils of McMurdo Station and Cape Evans. These soils were collected in 1961 and 1962. Dark brown pigmentation of the colony reverse which is marked on the potato sucrose agar is peculiar. The texture of the surface of the conidia is not conspicuously verrucose in young culture but is markedly so in mature conidia.

Dendryphiella salina (SUTHERLAND) PUGH et NICOT (Plate 1-A, D)

Trans. Brit. Mycol. Soc. **47**: 263, 1964
Syn. *Cercospora salina* SUTHERLAND

Growth on malt agar good, with aerial hyphae in fairly abundance, floccose in loose texture, 2-3 mm in height, olive-colored; conidial production scanty; reverse dark olive to almost black.

Growth on marine-water medium (0.1% yeast extract, 1.0% glucose and sea water) with adding of filter paper, rapid with fairly abundant production of conidia on filter paper. Conidiophores developed from vegetative hyphae, usually unbranched, bearing conidia terminally or subterminally, 2.5 μ or more in length, 4.0-4.5 μ in width, pale brown in color, paler to the apices. Conidia cylindrical, solitary or rarely in short chains, predominantly 4-6 septate, 20-45×6.5-9.0 μ; with longer 7-8-, rarely 9-11-septate conidia measuring 47-50×7.0-9.5 μ, usually straight, often slightly curved, pale brown colored.

Hab. Isolated from soils, Syowa Station (1956, 1960, 1961), soil and algae in East & West Ongul Island (1961), algae in Cape Evans (NA 64-1, 1962), soil of McMurdo Station (NA 62-1, 1962); a culture made by Dr. Y. KOBAYASI from the moss of McMurdo Station (Y. K., 1964).

The isolates varied in their ability to produce conidia on the media, but usually they sporulate very poorly on malt agar and potato agar. Good sporulation occurred on balsa-wood or filter paper either immersed in the sea-water medium or laid on the sea-water agar plate. Isolates obtained from the moss sporulated sparingly on all common media at first, but, after several transfers on sea-water agar, they tended to produce conidia on the dish edge at the periphery

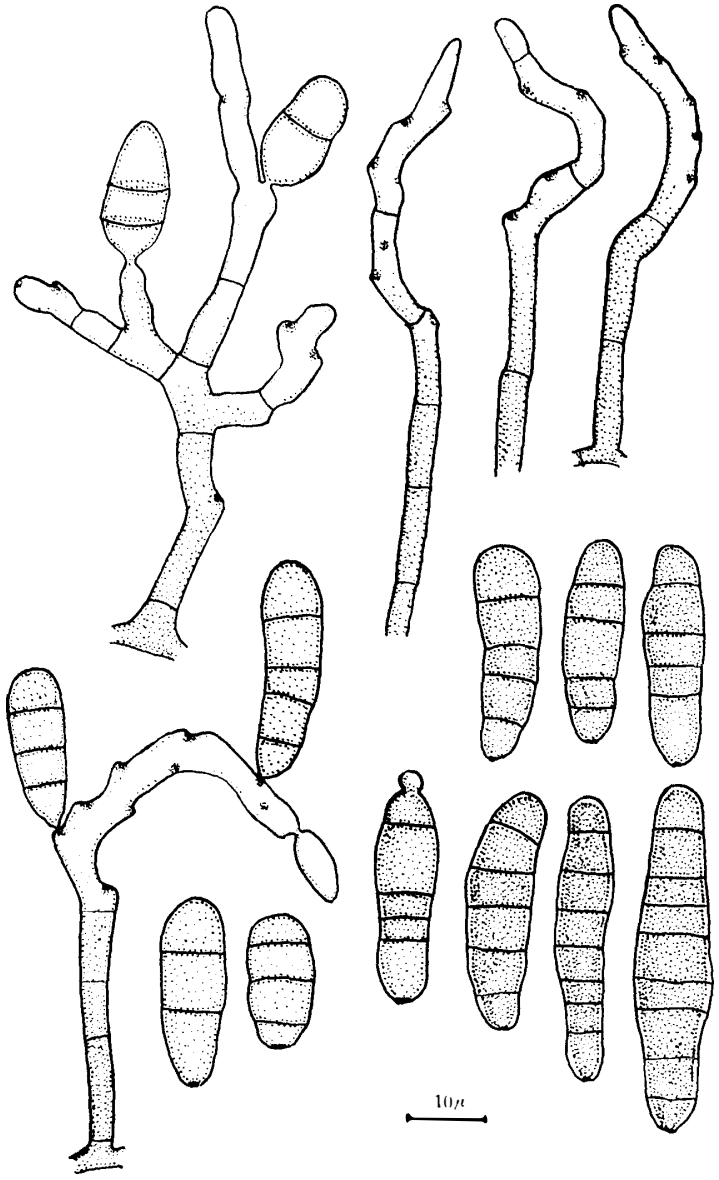

Fig. 1. Dendryphiella salina. Conidiophores and conidia.

of the colony on malt agar and on malt agar prepared with sea-water. Fortunately, by the courtesies of Dr. J. NICOT, Museúm National d'Histoire Naturelle, Paris, and of Dr. J. J. ELPHICK, Commonwealth Mycological Institute, I had an opportunity to examine typical cultures of *D. salina* isolated from *Salicornia* and others. Their microscopic characters agreed well with that of our antarctic strains in shape and diameter of the conidia. Our cultures are darker in colonial color and the production of the conidia is less than that of Dr. NICOT's and CMI's cultures. However, such differences did not seem to be distinctive characters separable from *D. salina*, so the present authors would include our strains in this species.

From the records, this species is distributed widely in intertidal regions (PUGH, 1962), on the rhizospheres of various plant (PUGH, 1960), on various seaweeds (SUTHERLAND, 1916), on wood blocks in the sea (JONES, 1962) and other places. Accordingly, it is reasonable that the present species was found from such seashore materials as soil, algae and mosses in Antractica because the coastal microorganisms of the Antarctica may easily be distributed widely in the continent and the regiones by its very rigorous climate.

Monodictys austrina TUBAKI, sp. nov. (Plates 1-B, 2-G, H)

Hyphis atro-brunneis, superficialibus vel submersus, irregulariter ramosae, 5-7 μ diam. Hyphis acrialis ramosae, 2-3 μ diam. Conidiophoris brevibus, simplicis, lateralibus, atrobrunneus, 2-3.5 μ diam. Conidiis atris, singularibus, acrogenis, sessilibus, cylindraceis, inaequalateralo-obpyriformibus vel curvatus, muriformibus, glabris vel asperatus, 27-45$\times$12-14 vel 38-60$\times$9-12 μ.

Vegetative hyphae dark brown, superficial or submerged, irregularly branched, 5-7 μ in diam.; aerial hyphae well developed, arise from vegetative hyphae, flexuous or staright, 2-3 μ in diam., pale brown. Conidiophores short when present, lateral, simple, concolours with the hyphae, with septa at bases, 2-3.5 μ in diam. Conidia sparingly produced on sea-water medium, the aleuriospore-type, on short conidiophores or directly from aerial hyphae; cylindrical, asymmetrical obpyriform or curved downward, with transverse and longitudinal septa, rough when matured, not or slightly constricted at septa; pale brown and opaque or subopaque at first, then becoming dark brown to fuscous; 27-45$\times$12-14 μ in obpyriform conidia, 38-60$\times$9-12 μ or more in cylindrical conidia.

Hab. Isolated from the soil of McMurdo Station, Antarctica, Dec., 1962 (NA 62-2; TYPE); from the culture-tube of unnamed algae collected in McMurdo Station, made by Dr. H. FUKUSHIMA, (NA-F). The type specimen and living culture derived from the type are deposited in the herbarium of the Institute for Fermentation.

The growth is rather good with fairly abundant olive-colored aerial mycelium. But, conidia are produced only on the marine water agar media (0.1% yeast extract, 1.0% glucose and 1.5% agar prepared with the natural sea water) with or without filter paper or on the balsa wood strips in the sea water containing 0.1% yeast extract. This indicates that the present fungus may be derived from the marine environment.

Conidia are at first not muriform, with only transverse septa, curved downward very often, and are considered the elongated or curved phaeophragmious spores. However, when matured, such conidia become muriform and mostly are asymmetrical obpyriform, dark brown to almost black in color. The color of the conidia is also unusual. The dark brownish pigment of the conidia may diffuse when put in water or Amann's fluid (pl. 2-G, H).

The present fungus is affined to *Monodictys pelagica* (JOHNSON) JONES, *Helicoma maritimum* LINDER and *Cirrenalia macrocephala* (KOHLMEYER) MEYERS et MOORE. *M. pelagica* is fairly close to the present fungus in the shape of conidia in maturity, but the young conidia do not curve, and, moreover, the conidia of the former species could not be succeeded in germination (JOHNSON, 1958). *Helicoma maritimum*

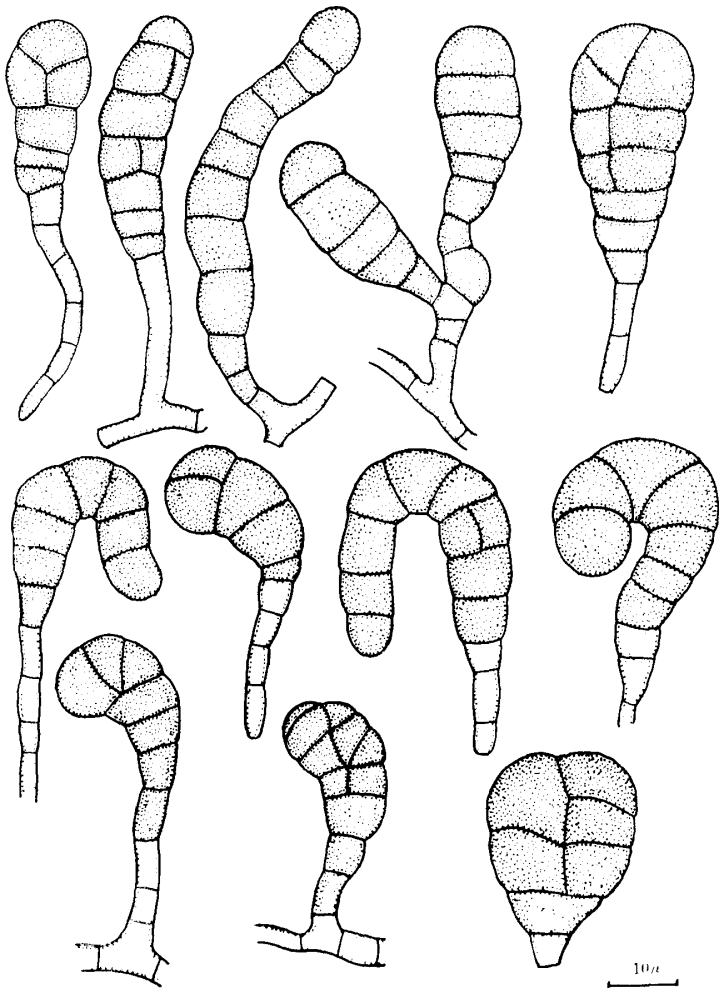

Fig. 2. Monodictys austrina. Conidiophores and conidia.

is also close to the present fungus, but its conidia tend to be more marked coiling than that of the present fungus. *Monodictys austrina*, therefore, differs remarkably from other species in both conidial morphology and character of conidial production on the sea-water medium. *Cirrenalia macrocephala*, originally described as a new species of *Helicoma* by KOHLMEYER (1958), nears the present fungus in somewhat helicoid conidia, but the conidia are phragmosporous, not muriform.

On the systematic position of this fungus, there was a problem whether it should belong to *Piricauda* or *Monodictys*. Before HUGHES' concept on the type species of *Piricauda* (1960), the present fungus seemed best to be assigned to *Piricauda* BUBÁK. However, HUGHES reported that the type species, *P. paraguayense*, has conidia in which the apical cell grows into a straight or curved septate, attenuated appendage, previously regarded as the conidiophore. As the conidia of the present fungus are exactly of the aleuriospore-type, the present authors believe that it is better placed in *Monodictys* S. J. HUGHES. The specific epithet, *austrina*, is derived from austrinus, suggestive of the southern.

Mucor mucedo (L.) BREFELD

ZYCHA, in Krypt. Fl. Mark Brandenburg VIa Pilze II, p. 85, 1935

Growth on malt agar after 4 days at 20°C rapid, attaining a diameter of 80 mm and up to 5 mm in height, silvery-gray in color, with pale yellowish submerged hyphae at central area and wide sterile margin; reverse colorless. Sporangiophores erect, unbranched, 27-40 μ in diam., with yellowish granular contents. Sporangia spherical, 100-170 μ in diam., at first yellow, then deep gray to almost black. Columellae cylindrical or oval, 60-100×50-70 μ. Spores ellipsoid or cylindric, 9-11×4-6 μ.

Hab. Isolated from the soil of McMurdo Sound, Dec., 1962 (NA 65-1).

This strain (NA 65-1) is peculiar in its maximum temperature for the growth. At 30°C, no growth was observed, and even at 25°C, its growth was very limited measuring only 5 mm in diam. without sporulation differing from the ordinary strain which grows very well at 25°C or even higher temperatures. At 25°C, however, it grew surprisingly well as described above. At 8°C, good growth was observed but slightly less than that at 20°C. At 5-6°C, the growth was very poor. Therefore, the temperature for the good growth may range between 10° and 20°C. This low-temperature organism is thought to be a fungus having special adaptability to unusually low temperatures and is thought to be distributed more widely in the Antarctic area.

Penicillium adametzi ZALESKI (Plate 3-J)

RAPER & THOM, the Penicillia p. 228, 1949

One strain (NA 65-2) was isolated from the soil of McMurdo Sound (1962). Growth on Czapek agar rapid, colony composed of velvety aerial mycelium and central area raised with slightly funiculose aerial hyphae; Russian Green or American Green to Varley's Green or Paris Green when matured. Reverse of the colony Cream Buff in color. Penicilli monoverticillate with short conidiophores measuring 30-50×2.5-3.0 μ. Conidia globose to subglobose, 2.5-3.5 μ, with delicately granulated surface. This strain fits closely to the present species in cultural and microscopical characters.

Penicillium canescens SOPP (Plate 3-K)

RAPER & THOM, the Penicillia, p. 316, 1949

One strain (NA 138-1), re-isolated from an agar-slant inoculated directly with the soil of East Ongul Island (1961), fits the description of RAPER & THOM (1949) in microscopical characters. Penicilli strongly divaricate with conspicuously roughened conidiophores, measuring 50-300 μ long or more. Conidia globose to subglobose, 2.5-3.0 μ in diam., roughened. The color of the colony reverse, although somewhat different from the description in Saccardo Umber to Snuff Brown at the center and Honey Yellow to Isabella Color at the margin; surrounding agar is hyaline to Olive Yellow or Light Yellowish Olive in color. The growth itself is restricted with radiate furrows, but not so buckled at the center.

Penicillium charlesii G. SMITH (Plate 3-L)

RAPER & THOM, the Penicillia, p. 248, 1949

Four strains (NA 17-1, NA 63-1, NA 67-2, NA 156-1) were isolated from the soils of the Syowa Station (1961), Cape Evans (1962), West Ongul Island (1961), McMurdo Station (1962). Growth after 3 weeks at 25°C rapid, attaining nearly 4-5 cm in diam. with velvety and radially furrowed surface. The central area more or less floccose, dark yellow-green shades close to Dark Green or American Green. Exudate lacking; odor not distinctive; reverse Olive-Brown or Fuscous in color; surrounding agar colorless. Penicilli monoverticillate, with simple or branched conidiophores measuring 30-150×1.8-2.0 μ; sterigmata mostly in 5-10 clusters, compact, 7.0-10×2.0-2.5 μ; conidia globose to subglobose, 2.0-3.5 μ in diam., smooth walled.

The gross morphological and faster growing characters closely fit the description and the typical culture of *P. charlesii* var. *rapidum* ABE (1956) which was later added to the present species by G. SMITH (1963) for the reason that the more rapid growth than that of the type is too trivial to be taken seriously.

Penicillium corylophilum DIERCKX (Plate 4-M)

RAPER & THOM, the Penicillia, p. 341, 1949

Two strains were isolated from the soil of Cape Evans (1962) and from the agar-slant inoculated directly with the soil of East Ongul Island. They closely fit the typical strain of *Penicillium corylophiloides* ABE which was presented as a new species of *P. corylophilum*-series. However, the name *P. corylophiloides* ABE was dropped by G. SMITH because it would be in the range of *P. corylophilum*.

Growth on Czapek agar restricted, colony with velvety and smooth or radially furrowed surface, dull bluish green near Bluish Gray-Green to Deep Bluish Gray-Green, becoming Celandin Green or Artemisia Green with age; exudate abundant in central area; reverse colorless. Penicilli typically bi-verticillate and asymmetrical. Conidiophores 120-190 μ long; conidia elliptical to subglobose, 2.5-3.5×2.0-2.5 μ, smooth.

Penicillium crustosum THOM (Plate 4-N)

RAPER & THOM, the Penicillia, p. 516, 1949

Three strains (NA 60-1, NA 63-1, NA 63-B) were isolated from the soil of of Cape Evans, McMurdo Sound (1962).

Growth on Czapek agar rapid, velvety, colony composed of radially furrowed aerial mycelium and with central flocculent area, grayish yellow green near Pea Green or Artemisia Green, becoming Mineral Gray in color. Crust-formation on the colony weak; exudate inconspicuous; odor earthy; reverse hyaline, pale cream to cinnamon-pink in age. Penicilli asymmetrical. Conidiophores coarse, 80-250 (500) μ long; sterigma 8-12×2.5-3.0 μ; conidia subglobose to ellipsoid, 3.2-4.0 (5.0) μ in diam., smooth.

TOLERANCE FOR CONCENTRATION OF SODIUM CHLORIDE

As already described, the relationships between the antarctic fungi and marine environment should be considered. Accordingly the cultures of *Dendryphiella salina* and *Monodictys austrina* were selected for the test and *Chrysosporium*-species were also treated under the control. Malt agar cultures were made to ascertain the maximum salinity at which the mycelial development occurred. Inoculation was made at 24°C for 8 days. Table 2 shows the results. In this table, it appeared that growth of all species occurred in 6% sodium chloride, and both *Dendryphiella salina* and *Monodictys austrina* are capable to grow in highly concentrated sodium chloride.

Table 2. Tolerance for sodium chloride.

Species	Conc. of NaCl	1	2	3	4	5	6	7	8	9	10
D. salina	NA 62-1	##	##	##	##	##	##	##	+	−	−
〃 〃	Y K	##	##	##	##	##	##	##	+	−	−
M. austrina	NA 62-2	##	##	##	##	##	##	±	−	−	−
Ch. pannorum		##	##	##	##	##	##	−	−	−	−
Ch. verrucosum		##	##	##	##	##	##	±	−	−	−

On the other physiological characters, the studies are now being carried out with other members of marine and coastal fungi isolated from the sea-water or the coast around Japan, and the data will be presented in the next paper on marine and coastal fungi.

CONCLUSION

From the Antarctic materials, collected in the McMurdo Sound area, at Cape Evans and other parts of adjacent mainland, a study of fungi was carried out in the Institute for Fermentation. Some fungi from the Syowa Station, East and West Ongul Island were also added. As a result, two specics of *Aspergillus*, five species of *Penicillium*, one species of *Mucor* and two species of *Chrysosporium* were identified. *Botryotrichum piluliferum* was isolated from the horse dung, left by E. SHACKLETON in Cape Royds in 1907-1909. *Dendryphiella salina*, a not uncommon coastal fungus, was isolated from many samples of soils, algae and mosses, and ecological relationships between the antarctic fungi and the marine environments were emphasized. A strain of *Mucor mucedo* was isolated, which showed a special character to grow well below 20°C, not at 25°C. The two strains assignable

properly to the genus *Monodictys* were isolated from the soil and the algae, and a new species, *Monodictys austrina*, was proposed on the basis of its isolation from the Antarctic materials.

We wish to express our thanks to Dr. Y. KOBAYASI, National Science Museum, and to Dr. H. FUKUSHIMA, Yokohama Municipal University, who collected sampls and provided the cultures for us. Some cultures were made by Mr. M. SONEDA, Nagao Institute, to whom we also express our thanks. Dr. M. B. ELLIS, Commonwealth Mycological Institute, gave us a valuable suggestion on the identification of *D. Salina* and Dr. J. NICOT, Museúm National d'Histoire Naturelle, and Dr. J. J. ELPHICK, CMI, sent us the typical cultures of it, to whom we are greatly indebted. We are indebted also to Dr. T. HASEGAWA, Director of the Institute for Fermentation, for his critisism on this work. Thanks are also due to Mr. T. ITO who was of major assistance in carrying out the experiments throughout the work.

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