Report of the Japanese Summer Parties in Dry Valleys, Victoria Land, 1963-1965☆

IV. Mycological Studies of the Antarctic Fungi

Part 2. Mycoflora of Lake Vanda, an Ice-Free Lake

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and Tetsuya TORII***

南極 Victoria Land の Dry Valley 調査報告

IV. 南極の菌類に関する研究

2. Vanda 湖の菌類相

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要　旨

南極 Victoria LandのDry ValleyにあるVanda湖の湖水と土壌の菌類相の研究を行なった．本研究の全供試試料は著者らの2人，鳥居鉄也および杉山純多によって，1964年12月から1965年1月の期間にVanda湖とその周辺て採取されたものてある．分離培地3種類(potato-glucose agar, Czapek's solution agar, glucose-glutamate agar)，その食塩濃度 (0, 30 g/*l*, 100 g/*l*)，分離温度2段階 (25 °C, 10 °C) という条件下で約200の菌株が分離された　湖の表層から湖底へいくに従い分離菌株数は増加する傾向が観察され，特に湖底の堆積物中からは，25 °Cの培養条件下て最も多くの菌株が分離されたVanda湖の表面から湖底までの温度と Cl^- 含量の変化を考慮すると，上の事実は興味深い．

Introduction

In 1964–1965 (Dec.–Jan.), T. Torii and J. Sugiyama were sent to the Dry Valleys, south Victoria Land, and to Ross Island, Antarctica, as members of the Second Victoria Land Research Expedition 1964–1965, supported by the National Science Foundation, U. S. A. and the Japan Polar Research Association, Japan.

☆ Field survey for this study (T. Torii–Geochemistry; J. Sugiyama–Biology) supported by the National Science Foundation, U. S A. and the Japan Polar Research Association, Japan

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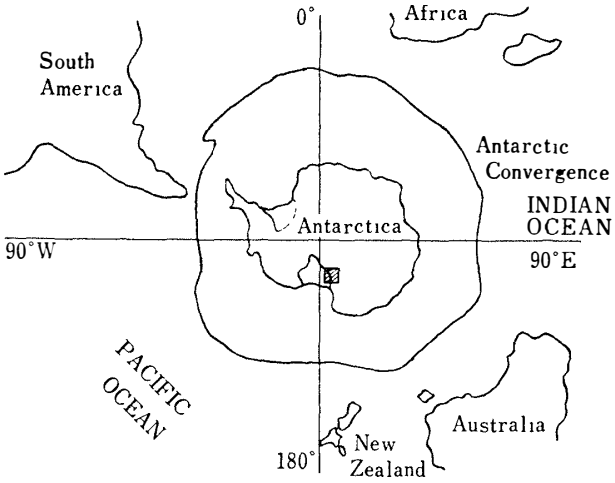

Fig. 1 Map of Antarctica and the surrounding continents

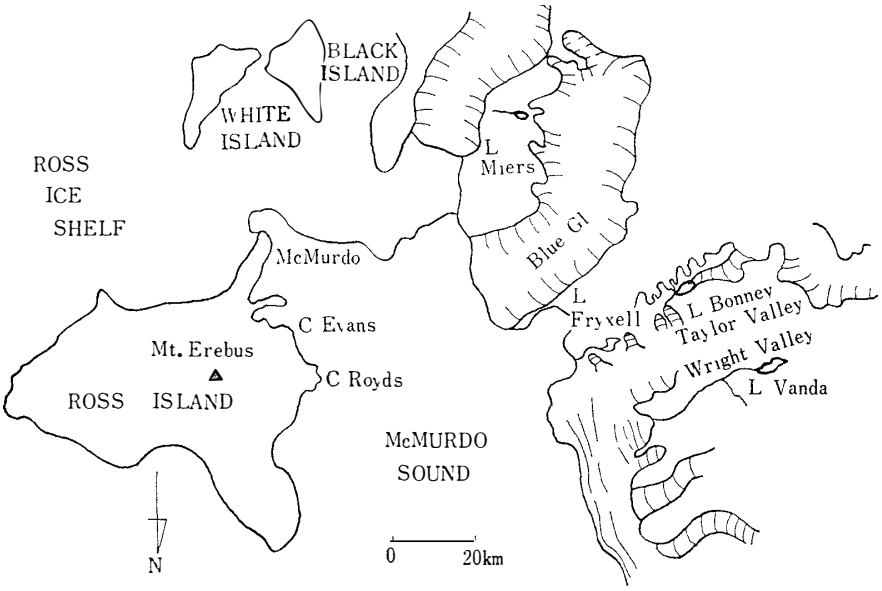

Fig 2 Map of the Dry Valley region, south Victoria Land, Antarctica.

The purpose of this expedition was to study the origin and evolution of the ice-free lakes in the Dry Valleys, Victoria Land, Antarctica, from geochemical as well as biological point of view. TORII *et al.* chiefly investigated geochemical aspects of these lakes (TORII *et al.**) ; while J. SUGIYAMA principally collected water and soil samples from the lakes and their surrounding territories for microbiological analyses. In the present paper, part of the mycological studies of materials from Lake Vanda (77°32′S, 161°30′E), one of the ice-free lakes in the Dry Valleys is

* Also accompanied were Tsurahide CHO (Physicochemist), Yoshio YOSHIDA (Geologist) and Zenkichi HIRAYAMA (See II. of this series)

Photo 1. Lake Vanda, Wright Valley, McMurdo Sound. Lake Vanda is perennially covered with ice about 3.5m thick. During the warm season ice at the margin of the lake thaws. (Photograph taken by Mr. Z. HIRAYAMA).

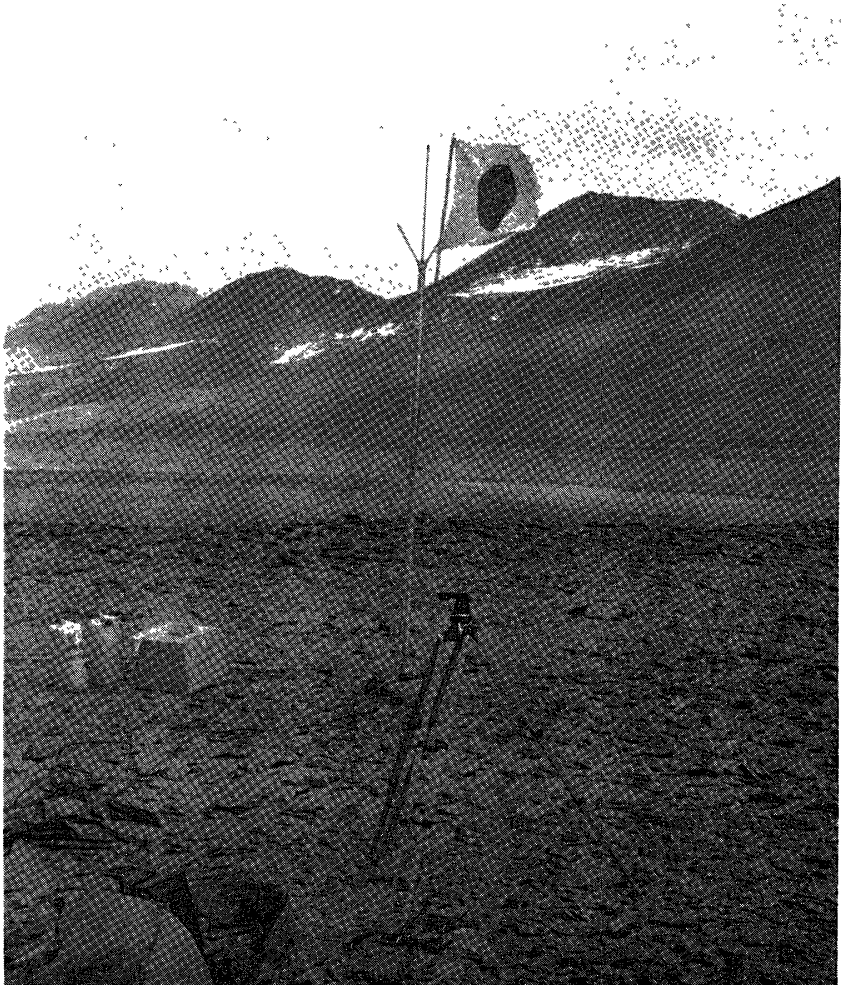

Photo 2. Lake Vanda, Wright Dry Valley. Profile of Wright Dry Valley showing the undrained bedrock surface. (Photograph taken by J. SUGIYAMA).

Photo 3. Aarrows indicate moss colonies at the margin of an outlet stream from Lake Miers. (Photograph taken by J. SUGIYAMA).

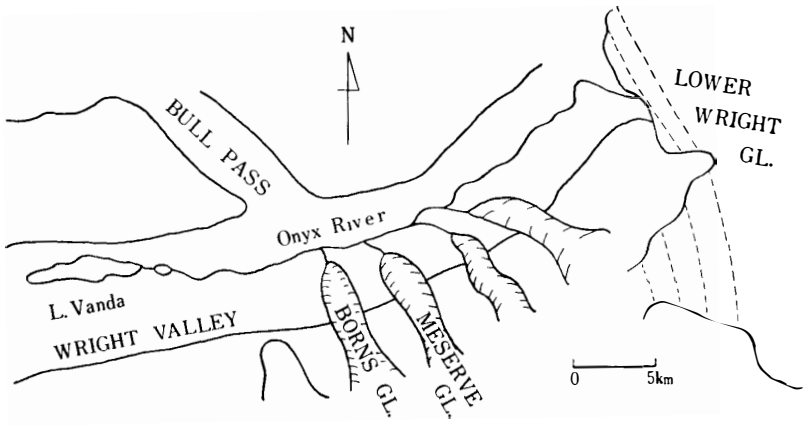

Fig. 3. Map of Lake Vanda, Wright Valley.

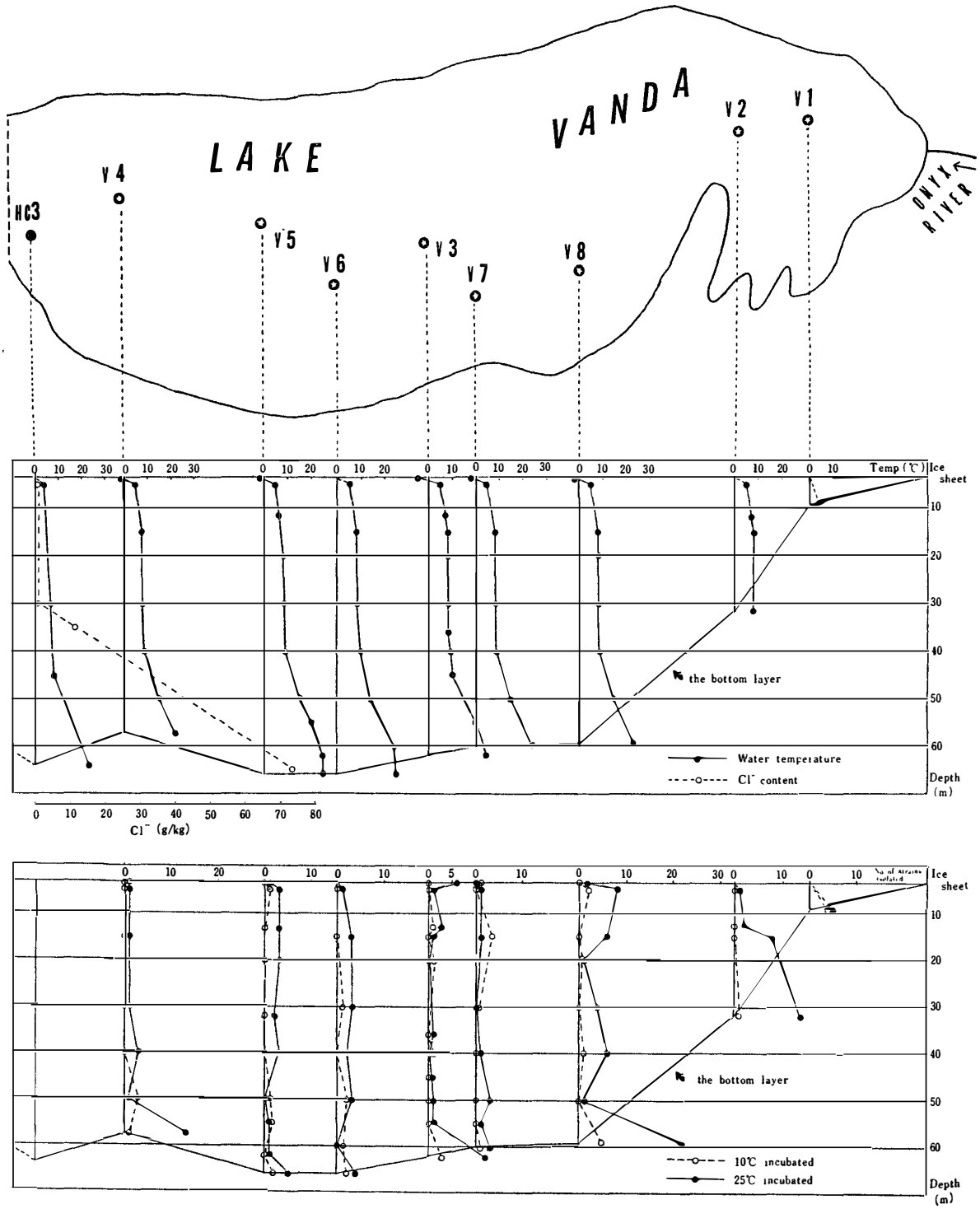

Fig. 4. Distribution of fungal strains in Lake Vanda, and fluctuations in water temperatures and Cl^- content.

presented. Some related ecological problems are also briefly discussed.

So far as is known, there is practically no information available at present concerning the fungi from the ice-free lakes, such as Lake Vanda (Figs. 1–3, Photos 1–3), Lake Bonney, Lake Miers, Lake Fryxell, etc., in the Dry Valleys, Victoria Land, Antarctica, except DI MENNA's papers which dealt with only yeast flora of McMurdo Sound (DI MENNA, 1960, 1966) and a paper by MEYER *et al.* (1962) with respect to microbiology, chiefly bacterial flora, of Pond Don Juan. Accordingly, this paper may represent the first detailed report as to mycoflora in such ice-free lakes in Antarctica.

Material and Methods

Water samples of the lakes were collected by using the KITAHARA's water sampler. All samples were transported by air in a week from the Antarctic to Tokyo. Isolation and counting of fungus cells were carried out by the plating method. Three kinds of media with different salt contents (0, 30 g/*l*, 100 g/*l*) were used: potato-glucose agar (PGA), CZAPEK's solution agar (CSA) and glucose-glutamate agar (GGA). All media were adjusted to pH 5.6 before sterilization. Cultures were incubated under two different temperatures, 10°C and 25°C, respectively.

Results and Discussion

The results obtained in the present investigation are all compiled in Tables 1, 2 and 3, and illustrated in Figs. 4 and 5. In Fig. 4 particularly, the number of fungal strains isolated from different water depths at eight different sampling stations (V1–V8), temperature ranges and Cl^- content (HC–3*) are demonstrated in order to examine whether or not there is any causal relationships between these environmental factors and distribution of the fungi in the lake and its surrounding areas.

Fungal genera discovered in this analysis are *Sporobolomyces*, *Candida*, *Trichosporon*, *Cryptococcus*, *Rhodotorula*, *Aspergillus*, *Penicillium*, *Stachybotrys* (V3, V4, both from the lake sediment) and *Trichoderma* (V5, V7). Among them, only species belonging to the genus *Aspergillus* and yeast were identified in this study**. Some other fungi referable to Actionomycetes, Phycomycetes, etc., were also isolated, but only the

* Temperature and Cl^- content in lake-water investigated on January 5, 1964 at a station HC–3 by TORII *et al.* (1965) (Fig. 4).

** The members belonging to the remaining groups are at present under investigation and the results will be published in our future papers.

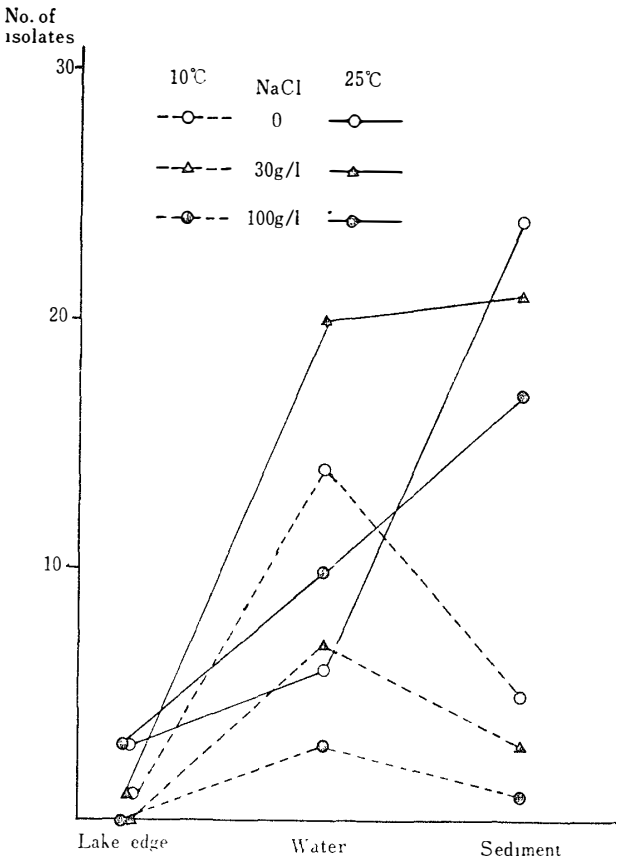

Fig. 5. Diagram illustrating numbers of isolates from three different samples, under varying conditions.

number of their strains was counted (Table 1).

Identified fungal species are as follows: *Sporobolomyces antarcticus* Goto, Sugiyama et Iizuka (sp. nov.)*, *Candida diffluens* Ruinen, *Can. scottii* Diddens et Lodder, *Can. torii* Goto, Sugiyama et Iizuka (sp. nov.)*, *Trichosporon byrdii* Goto, Sugiyama et Iizuka (sp. nov.)*, *Cryptococcus diffluens* (Zach.) Lodder et K.-van Rij, *Rhodotorula glutinis* var. *rufusa* Iizuka et Goto, *Rh. minuta* (Saito) Harrison, *Rh. rubra* (Demme) Lodder emend. Hasegawa; *Aspergillus flavus* Link**, the *Asp. glaucus* group, the *Asp. niger* group, *Asp. restrictus* G. Smith, *Asp. versicolor* (Vuill.) Tiraboshi.

It can be seen from this result (Table 1) that the three groups of fungi, notably *Aspergillus*, *Penicillium* and yeast, are dominant, attaining about 70 to 80 % of the strains isolated, in mycoflora of this sampling area.

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* These new species will be published in a separate paper (GOTO, S., SUGIYAMA, J. and IIZUKA, H., MS in preparation).

** Taxonomic account of *Aspergillus* species will be reported elsewhere (SUGIYAMA, Y. and IIZUKA, H. 1967).

Table 1. Fungi from water and soil samples of Lake Vanda and the Onyx River.

Localities	Fungi	Number of strains isolated	Identified	Sum total strains
Soil (the lake edge)	Penicillium	2	*Rhodotorula rubra*	
	Yeast**	1		
Soil (the lake edge)	Aspergillus*	1	*Aspergillus niger* group	9
	Other fungi	1		
Water (the Onyx River)	Aspergillus*	1	*Aspergillus niger* group	
	Penicillium	3		
Lake Vanda Surface Layer (just beneath the ice sheet) *ca.* 3.5m	Aspergillus*	4	*Aspergillus flavus*	
	Penicillium	3	*Asp. glaucus* group	
	Actinomycetes	2	*Asp. restrictus*	
	Other fungi	2		
5m	Aspergillus*	3	*Aspergillus niger* group	
	Yeast**	1	*Asp. versicolor*	
	Phycomycetes	1	*Cryptococcus diffluens*	
	Actinomycetes	3		
	Other fungi	1		
9m	Penicillium	1	*Rhodotorula glutinis* var. *rufusa*	
	Yeast**	1		
13m	Aspergillus*	4	*Aspergillus flavus*	
	Penicillium	4	*Asp. niger* group	
	Phycomycetes	1	*Asp. versicolor*	
	Other fungi	1		
15m	Aspergillus*	2	*Asp. flavus*	
	Penicillium	6		
	Actinomycetes	2		
	Other fungi	3		
20m	Penicillium	4		
	Phycomycetes	1		
	Other fungi	1		
30m	Penicillium	8		117
	Actinomycetes	3		
	Other fungi	2		
36m	Aspergillus*	1	*Asp. versicolor*	
40m	Aspergillus*	2	*Asp. flavus*	
	Penicillium	8	*Asp. glaucus* group	
	Actinomycetes	2		
	Other fungi	2		
45m	Other fungi	1		
50m	Aspergillus*	2	*Asp. flavus*	
	Penicillium	8	*Asp. niger* group	
	Yeast**	3	*Candida byrdii*, sp. nov.	
	Actinomycetes	1	*Rhodotorula minuta*	
	Other fungi	1	*Rhod. rubra*	
55m	Aspergillus*	2	*Asp. flavus*	
	Penicillium	3	*Asp. niger* group	
	Yeast**	1	*Cryptococcus diffluens*	
	Actinomycetes	1		
	Other fungi	1		
60m	Aspergillus*	1	*Asp. versicolor*	
	Penicillium	4		
	Actinomycetes	2		
	Other fungi	1		

Localities	Fungi	Number of strains isolated	Identified	Sum total strains
66m	Penicillium	2		
	Phycomycetes	1		
	Other fungi	1		
Sediment	Aspergillus*	14	*Asp. flavus*	78
	Penicillium	42	*Asp. niger* group	
	Yeast**	7	*Asp. restrictus*	
	Actinomycetes	1	*Asp. versicolor*	
	Other fungi	14	*Sporobolomyces antarcticus*, sp. nov.	
			Candida diffluens	
			Candida scottii	
			Rhodotorula rubra	

* Identified by Y. SUGIYAMA and IIZUKA (1967).
** Identified by GOTO, J. SUGIYAMA and IIZUKA (1967).

Table 2. Numbers of fungal strains isolated from Lake Vanda samples under varying conditions.

Origin	Total no. of strains cultured and examined	Temp. incu-bated	Salt content in medium (g/l)	PGA		CSA		GGA*		Total	
				No. of strains	%	No. of strains	%	No. of strains	%		
Soil and water samples from the lake edge and the Onyx River	8	10°C	0	1	12.5	0	0	0	0	1	
			30	0	0	0	0	—	—	0	1
			100	0	0	0	0	—	—	0	
		25°C	0	2	25.0	1	12.5	0	0	3	
			30	1	12.5	0	0	—	—	1	7
			100	2	25.0	1	12.5	—	—	3	
Water samples from the surface layer to 66m in depth	60	10°C	0	9	15.0	5	8.3	0	0	14	
			30	6	10.0	1	1.7	—	—	7	24
			100	2	3.4	1	1.7	—	—	3	
		25°C	0	5	8.3	1	1.7	0	0	6	
			30	13	21.7	7	11.5	—	—	20	36
			100	9	15.0	1	1.7	—	—	10	
Samples from lake sediments	71	10°C	0	5	7.0	0	0	0	0	5	
			30	3	4.2	0	0	—	—	3	9
			100	0	0	1	1.4	—	—	1	
		25°C	0	16	22.5	8	11.3	0	0	24	
			30	17	24.0	4	5.6	—	—	21	62
			100	12	17.0	5	7.0	—	—	17	

* Symbol (—) indicates that no test was made.

Table 3. Numbers of strains at each sampling station isolated under 10°C and 25°C.

Sampling station	Temperature incubated	
	10°C	25°C
V1	4	5
V2	1	21
V3	6	27
V4	5	21
V5	5	22
V6	6	16
V7	6	10
V8	10	38
Total	43	160

At all eight sampling stations, from V1 to V8, it was observed that there is a very clear trend in the zonal distribution of the strains, that is, the fungi are the largest in number in the sediment layers of the lake. In contrast, very few strains were isolated from water and soil samples collected in the Onyx River (an inflow stream) and the lake edge. However, this distribution pattern observed should be considered in the light of experimental conditions and natural ecological conditions in this very particular ice-free lake.

Numbers of the strains at eight different sampling stations, isolated under both 10°C and 25°C, are summarized in Table 3. Here, it can be seen that under 25°C incubation about four times as many fungal strains (attaining 160) as those incubated under 10°C (only 43) were isolated. Also, it was examined whether or not there is any relationship between the following factors, *i. e.*, (1) NaCl contents in culture media (0, 30 g/*l*, 100 g/*l*), (2) isolation temperatures (10°C, 25°C), (3) origin of materials, and (4) strain numbers isolated.

As a result of this cursory analysis, it was discovered that the temperature condition is a most likely factor determining the growth rate of at least some fungi from this ice-free lake; for there is evidently a trend that number of strains isolated is larger in sediment samples than in either water or soil samples when incubated under 25°C, regardless of NaCl content in culture media (Fig. 5, Table 2). It should be noted, on the other hand, that much less fungal strains were isolated when incubated under 10°C. This finding, *i. e.*, an outstanding increasing tendency in the number of fungal strains toward the lake bottom when isolated under 25°C, is extremely interesting, especially when considered in the light of temperature con-

ditions and Cl^- content measured in lake-water. Figures 4 and 5 very clearly demonstrate the relationship existent there.

A possible ecological relationship between Antarctic fungi and marine environment has been discussed already by TUBAKI (1961) and TUBAKI and ASANO (1965). They showed that *Dendryphiella salina* and *Monodictys austrina*, both isolated from antarctic soils, algae, and mosses, are capable to grow in highly concentrated sodium chloride (TUBAKI and ASANO, 1965). In the present study, it was demonstrated that many fungi were isolated from Lake Vanda materials with media containing very high content of sodium chloride. This fact may indicate that these fungi are well adapted to such a particular ecological condition; in fact, near the bottom in Lake Vanda actually salinity attains about 3.8 times as high as that in sea-water (WILSON and WELLMAN, 1962; TORII *et al.*, 1965). However, as pointed out already, the temperature of lake-water may be another important factor characterizing the distribution and habitat conditions of fungi in this lake.

As was already demonstrated well by various authors (see references), the "biological studies" of the Antarctic organisms, including all from the lower to the higher, with all possible synthetic approaches in earth science, are one of the most fascinating and much promising subjects, and we can expect to make a number of important discoveries and contributions, which no doubt will unravel and shed a light on the evolutionary background of the Antarctic continent.

Summary

1. Water and soil samples from Lake Vanda in the Dry Valleys, Victoria Land, Antarctica, were studied from the mycological standpoint. All material used in the present study was collected during December to January, 1964–1965, by two of the authors of the present paper, Tetsuya TORII and Junta SUGIYAMA, in Lake Vanda and its vicinity.

2. Under two different temperatures, 10°C and 25°C, using three different cultural media (potato-glucose agar, CZAPEK's solution agar and glucose-glutamate agar), each with three different salt contents (NaCl, 0, 30 g/*l*, 100 g/*l*), a number of fungal strains were isolated.

3. Isolated and identified fungi in this study are as follows: *Sporobolomyces*, *Candida*, *Trichosporon*, *Cryptococcus*, *Rhodotorula*, *Aspergillus*, *Penicillium*, *Stachybotrys* (V3, V4), and *Trichoderma* (V5, V7). Identified fungal species are as follows: *Sporobolomyces antarcticus* Goto, Sugiyama et Iizuka (sp. nov.), *Candida diffluens* Ruinen, *Can. scottii* Diddens et Lodder, *Can. torii* Goto, Sugiyama et Iizuka (sp. nov.), *Trichosporon byrdii* Goto, Sugiyama et Iizuka (sp. nov.), *Cryptococcus diffluens* (Zach.) Lodder et

K.-van Rij, *Rhodotorula glutinis* var. *rufusa* Iizuka et Goto, *Rh. minuta* (Saito) Harrison, *Rh. rubra* (Demme) Lodder emend. Hasegawa, *Aspergillus flavus* Link, the *Asp. glaucus* group, the *Asp. niger* group, *Asp. restrictus* G. Smith, and *Asp. versicolor* (Vuill.) Tiraboshi.

4. It was found that there is a gradually increasing tendency in the number and distribution of the fungal strains from the surface layer of the lake-water to the bottom—the lake sediment. In particular, the most numerous fungal strains were isolated from the bottom sediment of the lake when incubated under 25°C.

5. This fact is very interesting, considering the transitions in temperature and Cl^- content from the surface to the bottom layer in Lake Vanda.

Acknowledgements

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