A SURVEY OF TIDE-WASHED COASTAL AREAS OF SOUTHERN CALIFORNIA FOR FUNGI POTENTIALLY PATHOGENIC TO MAN

by

NINA DABROWA, M.A., Predoctoral Trainee in Mycology,
JOSEPH W. LANDAU, M.D., Assistant Research Dermatologist
VICTOR D. NEWCOMER, M.D., Professor of Medicine (Dermatology)
*ORDA A. PLUNKETT, Ph. D., Professor of Botany

Division of Dermatology, Department of Medicine, University of California, The Center for the Health Sciences, Los Angeles 24, California, U.S.A.

Medical Service, Veterans' Administration Service, General Medical and Surgical Hospital, Los Angeles 25, California, U.S.A.

and

**Department of Botany and Plant Biochemistry, University of California, Los Angeles 24, California, U.S.A.*

(14.VII.1964)

Fungi pathogenic to man have seldom been isolated from marine habitats although oceans are known to contain a wide variety of different fungi.[1] *Cryptococcus neoformans*,[2] several species of *Candida*,[2, 3] and some opportunistic fungi[1] have occasionally been isolated from marine environments. The exact role of oceans as a possible source of fungi pathogenic to man, however, has received little attention.

Ecologic studies have revealed the presence of pathogenic fungi in a variety of terrestrial environments.[4] The soil, particularly, has been recognized as a reservoir of those fungi responsible for most of the mycotic infections of man.[5–7]

The ability to grow and reproduce under laboratory conditions on culture media containing sea water is one of the characteristics

This study was supported in part by USPHS GRANTS A1-01478-07, 5 Tl Al 52-05 and 5Tl-AM-5265-05 and the Dermatologic Research Foundation of California, Inc.

of fungi capable of surviving in oceans.[1,8–20] *Blastomyces dermatitidis, Coccidioides immitis, Histoplasma capsulatum, Hormodendrum (Phialophora) compactum, Microsporon gypseum, Monosporium apiospermum, Sporotrichum schenckii* and *Trichophyton mentagrophytes* have also been demonstrated to possess this property.[11] In addition, *Candida albicans, C. immitis, H. capsulatum,* and *S. schenckii* have been found to remain viable in Seitz filtered sea water for as long as six weeks.[11]

The intertidal zone, an area extending from the low to the high water mark, is subjected at high tide to the stresses of the marine environment and at low tide to the stresses of the terrestrial environment. Although this area has not been considered as a source of fungous infections for man, the possibility exists.

This report presents the findings of a preliminary ecologic survey designed to determine the presence of fungi potentially pathogenic to man in the intertidal zone of beaches and on a tide-washed rookery. Various methods for the initial isolation of fungi from samples, including direct culturing on different media and inoculation into animals, are evaluated. In addition, the effects of distilled water, sea water and a solution of sodium chloride isotonic with sea water on the growth of some of the isolates are compared.

Materials and Methods

Samples of soils, algae (*Macrocystis* sp.), marine animals and feathers were collected at low tide from the intertidal zone at four Pacific Ocean beaches located near Los Angeles, California. These included the Carrillo, Point Mugu, Malibu and Palos Verdes Beaches. Each location was visited twice between April and October 1963. Similar samples were collected on one occasion in September 1963 from the intertidal zone at beaches of Santa Catalina Island. Samples of bird droppings were also obtained at this time from a rookery located offshore of Santa Catalina Island. All samples were placed in sterile paper containers, returned to the laboratory within several hours, and stored at 4° C for 24 to 48 hours until transferred to culture media. All marine animals were alive at the time of collection. These included sea urchins, sea anemones, bivalves and crabs, members of the phyla Echinodermata, Coelenterata, Mollusca, and Arthropoda, respectively. A sample is considered to include all material of one source collected on a trip to a site.

The test media used for primary isolation included Sabouraud medium (1% Bactopeptone[R], 4% dextrose, 2% agar) prepared with distilled water and with sea water and Mycosel[R] medium (1% phytone, 1% dextrose, 1.55% agar, 0.04% cycloheximide, 0.005% chloramphenicol) prepared in the same manner. Clear sea water, obtained from the littoral zone of Santa Monica Bay, California, was used within several weeks. The pH of the sea water

was 7.5 and the salinity 33 parts per thousand (3.3%) as previously determined.[11] Saline suspensions of more than 40 different samples were prepared by suspending approximately 50 grams of soil or smaller portions of algae, marine animals, or bird droppings in 100 ml solutions of physiologic saline with penicillin 5000 units per ml and streptomycin sulfate 1 mg per ml. The suspensions were vigorously agitated in sterile closed containers and aliquots directly inoculated on the four test media. Saline extracts to be used for animal injections consisted of the supernates resulting after eight of the suspensions were allowed to sediment for two hours. Four samples of feathers were inoculated directly on the four test media and also on autoclaved soil (121° C for 15 min at 15 lb per sq in.). Four samples of sea urchin spines were inoculated directly on the four test media. One soil sample from each site was baited with autoclaved human hair. All of these cultures used for primary isolation were incubated at room temperature and examined on alternate days for one month. New colonies at the time of their appearance were individually transferred to Sabouraud distilled water medium. The isolate of *Sporotrichum* was further subcultured on brain heart infusion agar (Difco) and incubated at 37° C. Species of *Candida* were identified by their morphology on cornmeal agar, growth in Sabouraud dextrose broth, and sugar fermentation reactions.[12]

Plates of Sabouraud distilled water medium were exposed to the air in the laboratory for approximately 20 minutes several times during this study.

Healthy female white mice weighing between 17 to 20 grams were divided into three groups. The mice in two of the groups were pretreated daily for four days with intraperitoneal injections of 0.5 mg and 1.0 mg of cortisone acetate, respectively. Five mice from each group were then inoculated intraperitoneally with 0.5 ml of a prepared saline extract. One additional mouse treated with the higher dose of cortisone and one mouse not treated were maintained as controls with each set of 15 inoculated mice. The animals were sacrificed after four weeks, autopsied, and portions of spleen, liver, kidney and lung were cultured on Sabouraud distilled water medium. These cultures were examined at weekly intervals for four weeks.

Salinity tolerance studies of 11 selected isolates were performed using Sabouraud medium with distilled water and with sea water as prepared for primary isolation. A third medium was prepared using a solution of sodium chloride dissolved in distilled water to a concentration of 3.4% to approximate the total solute content of sea water. These studies were performed, in duplicate, as previously described.[11]

RESULTS

One or more species of fungi were isolated from each of the saline suspensions of the samples with the exception of those of sea anemones, four of which were examined. The most species isolated from a single sample were 10 from bird droppings. A total of 60 isolates from different sources and sites were identified. This included 30 different species representing 21 genera. Four additional isolates produced only sterile mycelia. Most species present in one sample of a source from a site were also in the other sample

TABLE I

Fungi isolated from saline suspensions prepared from various sources obtained from tide-washed coastal areas of Southern California

A. Soil
- *Absidia* sp. 2 ab*
- *Alternaria tenuis* 2 cd
- *Alternaria* sp. 3 cd
- *Aspergillus fumigatus* 1 abc, 2 cd
- *Aspergillus nidulans* 4a
- *Aspergillus niger* 2 a, 3 ab
- *Aspergillus ustus* 1 ab
- *Hormodendrum* sp. 5 abc, 2 cd
- *Monosporium apiospermum* 3 cd, 5 b
- *Mucor* sp. 3a
- *Penicillium* sp. 2 bc
- *Rhodotorula* sp. 1 abcd, 2 abcd, 3 abcd, 4 abcd
- *Scopulariopsis brevicaulis* 1 cd, 2 cd
- *Sporotrichum schenckii* 3 cd
- *Syncephalastrum* sp. 2 ab
- *Trichoderma viride* 1 cd
- *Verticillium* sp. 2 bc

B. Echinodermata (sea urchins)
- *Cephalosporium* sp. 3 bc
- *Mucor* sp. 3b
- *Penicillium* sp. 4 bc
- *Syncephalastrum* sp. 3 ab
- *Trichosporon* sp. 3 abc
- *Verticillium* sp. 3 cd

C. Mollusca (bivalves)
- *Fusarium* sp. 3b, 4 ab
- *Geotrichum* sp. 4 ab
- *Rhodotorula* sp. 3 abcd
- *Trichoderma* sp. 4bc

D. Arthropoda (crabs)
- *Candida albicans* 3 abcd
- *Candida krusei* 3 abcd
- *Rhodotorula* sp. 3 abcd

E. Algae
- *Alternaria* sp. 3d
- *Cephalosporium* sp. 1a, 2 cd
- *Dematium* sp. 2 ab
- *Hormodendrum* sp. 1 cd, 3c
- *Hormodendrum* (*Phialophora*) *compactum* 2cd
- *Monosporium apiospermum* 5b
- *Mucor* sp. 1 ab
- *Scopulariopsis candida* 3 cd
- *Trichoderma viride* 3 cd

F. Bird droppings
- *Aspergillus fumigatus* 5a
- *Aspergillus niger* 5a
- *Candida albicans* 5 cd
- *Candida parapsilosis* 5 cd
- *Candida krusei* 5 cd
- *Cephalosporium* sp. 5b
- *Chrysosporium* sp. 5a
- *Paecilomyces* sp. 5 ab
- *Penicillium* sp. 5 ab
- *Verticillium* sp. 5b

*1 — 5 Sites from which samples were collected
1. Carrillo Beach
2. Point Mugu Beach
3. Malibu Beach
4. Palos Verdes Beach
5. Santa Catalina Island

*a — d Media on which fungus was isolated
a. Sabouraud distilled water medium
b. Sabouraud sea water medium
c. Mycosel^{R} distilled water medium
d. Mycosel^{R} sea water medium

of that source obtained at another trip to the site. These fungi, the sources and sites from which isolated, and the media on which the first isolate was cultured are presented in Table I.

Isolates from saline suspensions were cultured on 10 of the 15 possible combinations of test media as listed in Table II. There was no single medium on which all isolates were cultured and between 27 to 35 were cultured on each medium. Some plates of Sabouraud medium but none of Mycosel[R] medium were overgrown with bacteria. The combination of media on which the most isolates were cultured was the two preparations of Mycosel[R] medium and the combination on which the second most were cultured was the two preparations of Sabouraud medium. None of the fungi was initially cultured on only the two media prepared with sea water or the two with distilled water. All isolates including the sterile mycelia grew readily when transferred to Sabouraud distilled water medium regardless of the medium on which initially cultured.

TABLE II

Media on which fungi were isolated from saline suspensions

Combinations of media	Number of isolates
*abcd	8
abc	3
ab	11
bc	5
cd	18
a	7
b	6
c	1
d	1
Total number of isolates	60

*a—d media
a Sabouraud distilled water medium
b Sabouraud sea water medium
c Mycosel[R] distilled water medium
d Mycosel[R] sea water medium

The strain of *S. schenckii* converted completely to the yeast phase on brain heart infusion agar when incubated at 37° C. The strains of *C. albicans, Candida krusei* and *Candida parapsilosis* all fermented dextrose with production of acid and gas. *C. albicans* also similarly fermented maltose.

The fungi which have been identified by the same generic name were morphologically similar but were not necessarily identical.

Trichophyton terrestre was isolated on both the distilled water and the sea water preparations of Mycosel[R] medium from one of

the two samples of feathers obtained from Palos Verdes Beach. By employing direct inoculation onto autoclaved soil, *Arthroderma quadrifidum* was isolated from one of the two samples of feathers collected at Malibu Beach. This fungus converted to *T. terrestre*, the imperfect stage of *A. quadrifidum*, when transferred to Sabouraud medium.

Scopulariopsis brevicaulis and *T. terrestre* were both isolated from one of the two samples of sea urchin spines collected at Malibu Beach. Both fungi were cultured on the two preparations of Mycosel[R] medium and the latter was also cultured on Sabouraud distilled water medium. *T. terrestre* was isolated, in addition, from one of the two samples of sea urchin spines from Palos Verdes Beach. It was isolated on both preparations of Mycosel[R] medium and on Sabouraud distilled water medium. Neither *S. brevicaulis* nor *T. terrestre* was isolated from the saline suspensions of sea urchins.

No fungi were isolated from any of the soil samples baited with autoclaved hair.

Aspergillus sp., *Hormodendrum* sp. and *Penicillium* sp. were the only fungi isolated from air in the laboratory.

The fungi listed in Table III were isolated from the mice injected with saline extracts. Following passage through mice, a single

TABLE III

Fungi isolated from saline extracts by mouse inoculation

Saline extract*		Number of animals from which fungi were isolated			Fungus
Source	Site	Nontreated	2mg	4mg**	
A	1	1	1	1	*Scopulariopsis brevicaulis*
				1	*Aspergillus ustus*
A	2	1	0	2	*Rhodotorula* sp.
B	3	1	1	2	*Trichosporon* sp.
				1	*Cephalosporium* sp.
D	3	1	1	2	*Candida albicans*
				1	*Candida krusei*
E	2	0	0	1	*Streptomyces* sp.
		4	3	11	

*A—E Sources
A Soils
B Sea urchins
D Crabs
E Algae
*1—3 Sites
1 Carillo Beach
2 Point Mugu Beach
3 Malibu Beach
** Total dose of cortisone administered to each mouse prior to inoculation with the saline extract. Each extract was inoculated into three groups of five mice.

TABLE IV

The growth of fungi in Sabouraud media prepared with distilled water, sea water and 3.4 % sodium chloride

Fungus	Period of incubation in days	Source, site, media of initial isolate**	Distilled water medium		Sea water medium		3.4 % NaCl medium	
Alternaria sp.	7	A3cd	*5.5 × 6.0	33.0	*5.5 × 5.0	+27.5	*3.5 × 3.0	+10.5
			5.5 × 5.5	30.3	5.5 × 5.0	27.5	3.0 × 3.0	9.0
Aspergillus ustus	14	A1ab	7.0 × 6.5	45.5	2.5 × 3.0	7.5	2.0 × 2.0	4.0
			7.0 × 6.5	45.5	2.6 × 3.0	7.8	2.0 × 1.7	3.4
Aspergillus nidulans	14	A4a	4.0 × 3.0	12.0	2.0 × 1.5	3.0	4.0 × 3.5	14.0
			4.0 × 3.2	12.8	1.8 × 1.5	2.7	4.0 × 3.8	15.2
Cephalosporium sp.	14	E2cd	3.7 × 4.0	14.8	4.0 × 4.0	16.0	1.7 × 1.5	2.6
			3.5 × 4.0	14.0	4.0 × 4.2	16.8	1.5 × 1.5	2.3
Cephalosporium sp.	14	F5b	5.0 × 5.0	25.0	3.0 × 2.5	7.5	2.0 × 1.5	3.0
			5.0 × 5.0	25.0	3.0 × 3.0	9.0	1.7 × 1.6	2.7
Fusarium sp.	7	C4ab	6.0 × 5.5	33.0	6.0 × 6.0	36.0	3.5 × 3.5	12.3
			5.5 × 5.5	30.3	6.0 × 6.0	36.0	3.2 × 3.5	11.2
Hormodendrum sp.	14	A2cd	2.2 × 3.0	6.6	3.0 × 2.0	6.0	3.0 × 2.5	7.5
			2.2 × 2.8	6.2	2.5 × 2.5	6.3	3.0 × 2.8	8.4
Monosporium apiospermum	14	A3cd	2.5 × 2.5	6.3	2.5 × 1.7	4.3	2.5 × 1.8	4.5
			2.3 × 2.4	5.5	2.2 × 1.6	3.5	2.5 × 2.0	5.0
Scopulariopsis brevicaulis	7	A2cd	6.0 × 5.5	33.0	7.0 × 7.0	49.0	2.5 × 3.0	7.5
			6.0 × 6.0	36.0	7.5 × 7.0	52.5	2.5 × 3.0	7.5
Scopulariopsis candida	14	E3cd	2.0 × 2.5	5.0	6.5 × 6.5	42.3	4.5 × 5.0	22.5
			2.0 × 2.7	5.4	6.0 × 6.5	39.0	4.5 × 5.0	22.5
Sporotrichum schenckii	14	A3cd	3.5 × 3.0	10.5	2.3 × 1.8	4.1	1.2 × 1.0	1.2
			3.8 × 3.0	11.4	2.0 × 1.5	3.0	1.0 × 1.0	1.0

* — diameter of colonies in centimeters
\+ — mathematical product of diameters in square centimeters

** A—F Sources
- A Soils
- C Bivalves
- E Algae
- F Bird dropping

** 1—5 Sites
- 1 Carrillo Beach
- 2 Point Mugu Beach
- 3 Malibu Beach
- 4 Palos Verdes Beach
- 5 Santa Catalina Island

** a—d Media
- a Sabouraud distilled water medium
- b Sabouraud sea water medium
- c Mycosel^{R} distilled water medium
- d Mycosel^{R} sea water medium

species was recovered from each of two of the extracts and two species of fungi from each of three of the extracts. No fungi were obtained from mice inoculated with extracts from samples of soils and bivalves from Malibu Beach and of algae from Carrillo Beach. One fungus, a *Streptomyces* sp., was only isolated following animal passage, but all other isolates from animals were also recovered by culturing directly the saline suspension of the sample. More than 20 isolates were obtained by directly culturing suspensions which were not recovered from the animals injected with the corresponding extracts. Gross lesions were not observed in any of the animals. Positive cultures were obtained from the liver, spleen and kidneys, but none from the lungs of any of the animals. Eight different fungi were isolated from animals pretreated with the higher dose of cortisone, three from animals pretreated with the lower dose, and four from those not pretreated. No fungi were isolated from any of the control animals.

Colony diameters and their mathematical products of the selected isolates of fungi cultured to determine their relative growth on Sabouraud medium prepared with distilled water, sea water and 3.4% sodium chloride are presented in Table IV. The colonies of *Alternaria* sp., *Aspergillus ustus*, *Cephalosporium* sp. (bird dropping isolate), and *S. schenckii* were largest on distilled water medium and smallest on sodium chloride medium. Colonies of *Cephalosporium* sp. (algae isolate), *Fusarium* sp. and *S. brevicaulis* were largest on sea water medium and smallest on sodium chloride medium. The sequence of media demonstrating largest to smallest size of colonies was different with each of the four remaining fungi. No correlation was apparent between the salinity tolerance of a fungus and the medium or media on which it was isolated.

Discussion

Many of the fungi isolated in this study are species known to be pathogenic to man. *H. compactum*, *M. apiospermum*, *S. brevicaulis*, *S. schenckii* and species of *Aspergillus*, *Candida*, and *Geotrichum* produce a variety of cutaneous, subcutaneous and systemic infections[4]. Species of *Candida*, *Aspergillus* and *Mucoraceae* (*Absidia* and *Mucor*) are the most common etiologic agents of disseminated mycotic infections in patients with serious debilitating diseases.[13] Species of *Rhodotorula* have infrequently been associated with human cutaneous and systemic infections.[24] Other fungi such as species of *Cephalosporium*, *Fusarium*, *Penicillium* and *Trichosporon* have been cultured from patients with keratomycosis.[15] *T. terrestre*, the only fungus isolated belonging to a recognized dermatophyte genus, is currently considered to be non-pathogenic and a common soil inhabitant.[6]

Most of the isolated fungi are included among the Fungi Imperfecti although a few belong to the class Ascomycetes or class

Phycomycetes. The cultural conditions, however, were purposely selected to favor the growth of human pathogenic fungi, most of which are members of the class Fungi Imperfecti and the distribution probably does not represent the over-all fungal flora of the intertidal zone. Ascomycetes and aquatic Phycomycetes have frequently been isolated from marine waters in studies utilizing cellulose baiting and liquid media.[1, 8] Actinomycetes are also found in this habitat[16] but their growth may have been suppressed, in some instances, by the penicillin and streptomycin contained in the saline suspensions. The production of antifungal substances by algae[17] and marine bacteria[18] may also have influenced the type of fungi recovered.

Several possible reasons may explain why some of the fungi were initially isolated on one medium and not on another. Cycloheximide, a constituent of Mycosel[R] medium, is added for the purpose of suppressing the growth of some fungi including species of *Aspergillus, Mucor, Paecilomyces, Penicillium* and *Trichoderma*.[19] This permits the isolation of organisms that might otherwise be obscured by the more rapidly growing fungi. Chloramphenicol, in a similar manner, suppresses bacterial growth. Mycosel[R] medium and Sabouraud medium also differ in their sources of nitrogen and concentrations of dextrose. The presence or absence of sea water in a medium did not appear to be particularly important since all isolates grew readily when transferred to medium containing distilled water. The omission of any single medium would have resulted in the failure to recover at least one fung s from a sample. This observation, however, may be more closely related to availability of an additional medium for culturing an organism present in low concentrations rather than to any specific property of the medium.

The isolation of a fungus from a sample obtained from the intertidal zone does not necessarily imply that it was indiginous to the area. All of the fungi identified in this investigation have been isolated previously from terrestrial habitats and most of them also from marine habitats. *M. apiospermum* and *S. schenckii* have not been isolated from marine sources but other species of both of these genera have been cultured from oceans.[1] *C. albicans* has not been recovered from open ocean waters but has from estuarine water and sediments[2] of Southern California and from the intestines of sea gulls[20] in Baja California. *C. albicans* is not well-established as a component of the mycotic flora of soil, but it has occasionally been recovered from this source.[5]

No specific criteria are available to distinguish precisely between marine and terrestrial fungi.[1, 10, 16] A fundamental feature of the marine environment is its relatively uniform salinity.[1] Sodium chloride constitutes 86% of the total solids dissolved in sea water. Marine fungi can be categorized as either stenohaline, tolerant only to small magnitude changes in salinity, or euryhaline, tolerant

to a wide range of salinity.[1, 10] None of the fungi recovered in this study can be considered as stenohaline. An absolute requirement for either sea water[21] or sodium ion[22] in the media used for isolation has been proposed as a characteristic of marine bacteria. Many fungi, however, have been isolated from oceans that grow well on media prepared with either sea water or distilled water.[8, 9, 16, 23, 24] Seventy-five per cent of the isolates from samples collected from the intertidal zone in this study were initially cultured on both media with sea water and media with distilled water.

The growth of fungi cultured on media containing distilled water has been compared with growth on media containing sea water or sodium chloride. Observations of this type indicate that generally fungi isolated from oceans grow better on the latter media as measured by colony diameter or mycelial weight.[1, 8–10] Smaller colony size was observed with eight human pathogenic fungi when their growth on sea water media was compared to that on distilled water media.[11] Colonies of four of the 11 fungi examined in this study were larger on sea water media than on distilled water media. Further information, however, is required before such salinity tolerance studies can be interpreted. Some marine isolates[8, 9] grow optimally on distilled water medium and some terrestrial isolates[23] grow best on sea water medium. Temperature and nutrition, in addition, influence the salinity tolerance of fungi.[23, 25, 26]

Human isolates of two of the fungi examined in the salinity tolerance tests were previously studied. The relative sizes[11] of colonies of the human isolates of *S. schenckii* and *M. apiospermum* were approximately the same as those cultured from the intertidal zone.

The growth of fungi on media containing 3.4% sodium chloride which is isotonic with sea water, did not parallel its growth on sea water medium whereas in a previous study the colony size of seven of eight human pathogenic fungi was less on media prepared with 3.4% sodium chloride than on sea water media.[11] The substitution of certain osmotically active materials isotonic with sea water, other than sodium chloride, has been observed to result in a pattern of growth similar to that obtained with media prepared with artificial sea water.[26] The constituent in sea water responsible for enhancement or inhibition is not solely sodium chloride and is probably related, at least in part, to other salts.[1, 27] The effect of the addition of sodium chloride to a culture medium on the growth of a fungus is dependent on properties intrinsic to the particular organism.

Strains of a fungus isolated from a marine environment do not, in general, exhibit distinctive metabolic differences from terrestrial strains.[2, 24] Marine isolates of *C. parapsilosis*, however, may not exhibit the usual fermentation reactions when isolated,[2, 3] and may require a period of adaptation on organically rich media.[2] The strains of *Candida* isolated in this study produced characteristic fermentation reactions.

The primary objective of this investigation was to determine the possible presence of pathogenic fungi located in the intertidal zone. No attempt was made to determine if the fungi were transients or intimately associated biologically as pathogens or saprophytes of the marine plants and animals. Fungi have been recognized as active agents in the destruction of living and non-living marine plant and animal materials.[1] Transient fungi may also have been deposited by either bathers, animals, terrestrial run-offs, or air currents. It is unlikely that the fungi cultured represented air contaminants originating in the laboratory since only a few species were isolated on exposed culture plates during the technical procedures.

Techniques employing soil supplemented with a keratinaceous bait have proved useful for the isolation of keratinophilic fungi.[6,28,29] *A. quadrifidum,* the perfect stage of *T. terrestre,* which was in this study cultured from feathers inoculated onto autoclaved soil, was first isolated and identified in 1961 from soils baited with autoclaved hair.[28] This fungus has also been recovered by baiting with autoclaved wool coastal soils collected from sites near bird traps.[29] Further studies of the possible relationship between *A. quadrifidum* and birds are indicated.

The use of laboratory animals has become a valuable adjunctive procedure for the isolation of pathogenic fungi from soils.[5, 7] Comparison of the mouse inoculation, direct plating, and hair baiting methods has revealed that some fungi may be recovered by one of these techniques but not by another.[5] In the current investigation only one organism was recovered from mice that was not also isolated by direct plating of the sample while a number of organisms were isolated by direct plating that were not recovered from animals. The advantage of cortisone treated compared with untreated mice for the isolation of fungi has previously been observed in the screening of soils.[7]

Although *C. immitis,* a fungus endemic to southwestern United States was not isolated in this study, the possibility of its presence in the intertidal zone deserves further consideration. *C. immitis* is generally found in arid and semi-arid areas of the Lower Sonoran Life Zone[30, 31] but under laboratory conditions it survives in sea water,[11] in solutions of sodium chloride to saturation,[11, 32] and in a variety of soils from different regions.[30] Furthermore an association between an increase in the soluble salts in soils, particularly sodium and chloride, and the presence of *C. immitis* has been suggested.[31] Soils from which *C. immitis* has been isolated, contained 8 to 75 times more sodium and 10 to 240 times more chloride than soils not yielding *C. immitis.*[31] The highest concentration of sodium and chloride in the soils with *C. immitis,* however, was only approximately one-half that in sea water.

The demonstration of potentially pathogenic fungi in samples from five coastal areas of Southern California suggests that certain

fungous infections may be acquired at such sites. Substantiation of this speculation, however, must await additional epidemiologic investigations.

Summary

Samples of soils, marine animals, algae, feathers and bird droppings were collected from tide-washed coastal areas of Southern California. Thirty-three different species representing 24 genera of fungi were isolated. These included well-recognized and potentially pathogenic fungi such as *Aspergillus fumigatus, Candida albicans, Candida krusei, Candida parapsilosis, Hormodendrum (Phialophora) compactum, Monosporium apiospermum, Scopulariosis brevicaulis* and *Sporotrichum schenckii*. Other less common potential pathogens were species of *Absidia, Aspergillus, Cephalosporium, Fusarium, Geotrichum, Mucor, Penicillium, Rhodotorula* and *Trichosporon*. Sabouraud medium and Mycosel[R] medium prepared with distilled water and with sea water were employed to isolate these fungi from saline suspensions of the samples. Some fungi were isolated on one medium and not on another but all subsequently grew satisfactorily when subcultured on Sabouraud distilled water medium. The colony sizes of four of 11 fungi examined in salinity tolerance studies were larger on sea water media than on distilled water media. Growth on media containing a solution of sodium chloride isotonic with sea water did not parallel growth on sea water media. *Streptomyces* sp. was the only fungus recovered from saline extracts inoculated into laboratory mice which was not also isolated by the direct culturing of saline suspensions of the samples. *Trichophyton terrestre*, a non-pathogenic member of a dermatophyte genus, was cultured from feathers and sea urchin spines inoculated directly on solid media. *Arthroderma quadrifidum*, the perfect stage of *T. terrestre*, was isolated from feathers inoculated on autoclaved soil. These findings suggest that the intertidal zone may constitute an additional reservoir of potentially pathogenic fungi and that certain fungous infections may be acquired by exposure in these areas.

References

1. JOHNSON, T. W., JR. & SPARROW, F. K., Jr. Fungi in oceans and estuaries, 668 pp. Weinheim, J. Cramer, 1961.
2. FELL, J. W. & VAN UDEN, N. Yeasts in marine environments. pp. 329—340. in OPPENHEIMER, C. H. Symposium on Marine Microbiology. Springfield, Charles C. Thomas, 1963.
3. PHAFF, H. J., MRAK, E. M. & WILLIAMS, O. B. Yeasts isolated from shrimp. Mycologia, 1952, **44**: 431—451.
4. EMMONS, C. W., BINFORD, C. H. & UTZ, J. P. Medical Mycology, 380 pp. Philadelphia, Lea & Febiger, 1963.
5. MCDONOUGH, E. S., AJELLO, L., AUSHERMAN, R. J., BALOWS, A., MCCLELLAN,

J. T. and BRINKMAN, S. Human pathogenic fungi recovered from soil in an area endemic for North American Blastomycosis. Amer. J. H.yg., 1961, **73**: 75—83.
6. AJELLO, L. Present day concepts of the dermatophytes. Mycopathol. et Mycol. Appl., 1962, **17**: 315—324.
7. BUSAILAH, L. & EVENSON, A. E. The use of cortisone-treated mice in the screening of soil for pathogenic fungi. Mycopathol. et Mycol. Appl. 1962, **17**: 293—298.
8. BARGHOORN, E. S. & LINDER, D. H. Marine fungi: their taxonomy and biology. Farlowia, 1944, **1**: 395—467.
9. GUSTAFSSON, U. & FRIES, N. Nutritional requirements of some marine fungi. Physiol. Plant., 1956, **9**: 462—465.
10. RITCHIE, D. The evolution of salinity tolerance in fungi. Trans. N. Y. Acad. Sci., 1960, **23**: 138—140.
11. DZAWACHISZWILI, N., LANDAU, J. W., NEWCOMER, V. D. & PLUNKETT, O. A. The effect of sea water and sodium chloride on the growth of fungi pathogenic to man. J. invest. Derm. 1964, **43**: 103—109.
12. AJELLO, L., GEORG, L., KAPLAN, W. & KAUFMAN, L. Laboratory Manual for Medical Mycology. Atlanta, U. S. Department of Health, Education and Welfare, Public Health Service, 1962.
13. LANDAU, J. W., NEWCOMER, V. D. & SCHULZ, J. Aspergillosis. Report of two instances in children associated with acute leukemia and review of pertinent literature. Mycopathol. et Mycol. Appl., 1963, **20**: 177—224.
14. SHELBOURNE, P. F. & CAREY, R. J. *Rhodotorula* fungemia complicating staphylococcal endocarditis. J.A.M.A., 1962, **180**: 38—42.
15. GINGRICH, W. D. Keratomycosis. J.A.M.A., 1962, **179**: 602—608.
16. GREIN, A. & MEYERS, S. P. Growth characteristics and antibiotic production of Actinomycetes isolated from littoral sediments and materials suspended in sea water. J. Bact., 1958, **76**: 457—463.
17. WELCH, A. M. Preliminary survey of fungistatic properties of marine algae. J. Bact. 1962, **83**: 97—99.
18. BUCK, J. W., AHEARN, D. G., ROTH, F. J. Jr. & MEYERS, S. P. Inhibition of yeasts by a marine bacterium. J. Bact., 1963, **85**: 1132—1135.
19. GEORG, L. K., AJELLO, L. & GORDON, M. A. A selective medium for the isolation of *Coccidioides immitis*. Science, 1951, **114**: 387—389.
20. VAN UDEN, N. Factors of host yeast relationship. pp. 635—643 in GIBBONS, N. E.: Recent Progress in Microbiology. Toronto, University of Toronto Press, 1963.
21. ZOBELL, C. D. Marine Microbiology. p. 121. Waltham, Chronica Botanica Co., 1946.
22. MACLEOD, R. A. & ONOFREY, E. Studies on the stability of the Na^+ requirement of marine bacteria. pp. 481—489. In OPPENHEIMER, C. H. Symposium on Marine Microbiology Springfield, Charles C. Thomas, 1963.
23. RITCHIE, D. The effect of salinity and temperature on marine and other fungi from various climates. Bull. Torrey Bot. Cl., 1959, **86**: 367—373.
24. AHEARN, D. G., ROTH, F. J. Jr. & MEYERS, S. P. A comparative study of marine and terrestrial strains of *Rhodotorula*. Canad. J. Microbiol., 1962, **8**: 121—132.
25. TE STRAKE, D. Estuarine distribution and saline tolerance of some Saprolegniaceae. Phyton., 1959, **12**: 147—152.
26. RITCHIE, D. & JACOBSOHN, M. K. The effects of osmotic and nutritional variation on growth of a salt-tolerant fungus, *Zalerion eistla*. pp. 286—299. In OPPENHEIMER, C. H. Symposium on Marine Microbiology. Springfield, Charles C. Thomas, 1963.
27. GRAY, W., PINTO, P. V. C. & PATHAK, S. G. Growth of fungi in sea water medium. Appl. Microbiol., 1963, **11**: 501—505.
28. DAWSON, C. O. & GENTLES, J. C. The perfect states of *Keratinomyces ajelloi* VANBREUSEGHEM, *Trichophyton terrestre* DURIE & FREY and *Microsporum nanum* FUENTES. Sabouraudia, 1961, **1**: 49—57.
29. POUGH, G. J. F. & MATHISON, G. E. Studies on fungi in coastal soils III. An ecological survey of keratinophilic fungi. Trans. Brit. mycol. Soc., 1962, **45**: 567—572.

30. LUBARSKY, R. & PLUNKETT, O. A. Some ecological studies of *Coccidioides immitis* in soil. pp. 308—310. In STERNBERG, T. H. & NEWCOMER, V. D. Therapy of Fungus Diseases. Boston, Little Brown and Co. 1955.
31. EGEBERG, R. O. Factors influencing the distribution of *Coccidioides immitis* in soil. pp. 652—655. In GIBBONS, N. E. Recent Progress to Microbiology, Toronto, University of Toronto Press. 1963.
32. FRIEDMAN, L., SMITH, C. E. & BERMAN, R. J. Studies on the survival characteristics of the parasitic phase of *Coccidioides immitis* with comments on contagion. Amer. Rev. Resp. Dis., 1962, **85**: 224—231.