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Filamentous Fungal Populations of Hawaiian Beaches[1]

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ABSTRACT: Heterotrophic micro-organisms were studied on three Hawaiian beaches—two of volcanic origin and one of carbonate. The volcanic beaches consisted of coarse particles with little organic matter. The carbonate beach consisted of coarse-to-fine, light-colored particles and contained more organic material than the volcanic sands. Fungi populations of the three beaches differed noticeably in their tolerance to temperature, salinity, and pH. In vitro testing of selected fungi showed wide tolerance to salinity levels, less tolerance to the high temperature of black sand, and no adaptation to alkaline pH levels. Heterotrophic microbe populations were greatest in the supratidal zone, except for the intertidal bacterial population of the black sand beach. In the subtidal black zone of the carbonate beach, only bacteria were well established, actinomycetes were absent, and fungi were few. Fifty percent of the fungi were common to any two of the three beaches. Zonal decrease in numbers at all three beaches was attributed to differences in submergence time.

HAWAIIAN BEACHES DIFFER IN origin. Carbonate beaches of foraminiferal origin predominate on the older islands. On Hawaii, the Hawaiian island most recent in origin with currently active volcanoes, there are also beaches of volcanic mineral sand. Volcanic black sand is an acidic basalt; volcanic green sand is an alkaline olivine. Beaches vary in sand type, organic content, pore water, salinity, pH, and temperature. The beaches may select microbial populations for tolerance to beach conditions similar to the selection of microbial populations for tolerance of soil types.

Studies of fungi in the marine beach habitat have dealt largely with the shoreline of continental land masses in temperate climates with more attention to geographical distribution of microbes than to their ecology. Brown (1958) investigated the pH optima of English beach fungi and Westheide (1968) considered the ecology of a North Sea beach heterotrophic community. Investigations of fungi in the marine environment of tropical and subtropical Pacific islands are few (Johnstone 1947, Sparrow 1948, Steele 1967, Kohlmeyer 1969, Kishimoto and Baker 1969, Anderson 1979, Dunn and Baker 1983). Emphasis in these studies ranged from macrofungi to phycomycetes, actinomycetes, and human pathogens, with emphasis on their geographical distribution. Little regard has been given to the role of microfungi in the beach habitat (Sparrow 1937) except for the study of Enewetak Atoll beaches (Dunn and Baker 1983).

There is a group of pyrenomycetes which is generally found on wood and algae drift material in marine habitats, including Hawaii. These are traditionally thought of as marine fungi. The marine fungi were not selectively sampled in this study, although they are present and one was a frequent isolate. Although this study was directed toward the fungi, the bacteria and actinomycetes were also sampled.

MATERIALS AND METHODS

Sample Sites and Sampling

Three leeward beaches on two of the Hawaiian Islands were selected: (1) Punaluu

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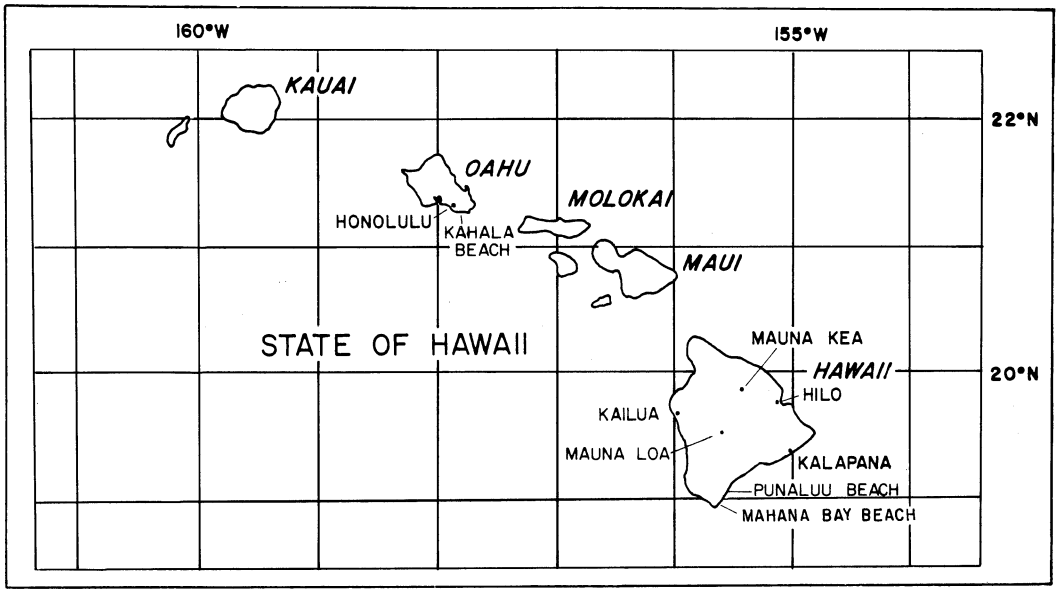

FIGURE 1. Mahana Bay and Punaluu Beaches, Island of Hawaii, and Kahala Beach, Oahu, sites of a study of heterotrophic micro-organisms.

Beach, Island of Hawaii, an acidic basalt beach at the southernmost point of the Hawaiian Islands; (2) Mahana Bay Beach, Island of Hawaii, an alkaline, olivine volcanic beach; and (3) Kahala Beach, Island of Oahu, an alkaline, carbonate sand beach of foraminiferal origin, and the only one of the three protected by a fringing reef (Figure 1). Sand samples were taken along transects through the tidal zones. Zones were designated as follows: (a) land, above the vegetation line but not in the vegetation; (b) supratidal, the top of the beach extending inward to the vegetation; (c) intertidal, area of normal tidal fluctuation; (d) subtidal, for samples from −1 m below sea level inshore to −10 m off the beach, and (e) the black zone, under the subtidal sand.

Sand from the three beaches was sampled in June and October of 1971, and again in January, April, and July of 1972. Sand from zones above water was collected with sterile implements at low tide. The sampling depth was surface to 3 cm after the removal of a thin surface layer. In subtidal areas, sand was collected with a 25-mm (inside diameter) sterile glass core sampler, after removal of the upper aerobic sand layer. Samples were stored in sterile "Whirl-Pak" bags (NASCO Whirl-Pak Hydro Products Co., San Diego, Calif.). Subtidal samples were decanted before sealing. All data from the various sampling dates were combined by zones.

Analytical Procedures

The oxygen content of water was measured with a Precision galvanic cell oxygen analyzer, or by the Winkler method (Strickland and Parsons 1968). Oxygen contamination, during sampling in the black zone, was reduced by use of a 50-ml syringe fitted with a 15-cm 16-gauge needle to withdraw the water sample with minimal disturbance to the sand above. Salinity measurements were made with an American Optical Co. Goldberg temperature-compensated salinity refractometer. Temperature was measured in the upper 5 mm of sand.

Organic carbon was estimated by drying samples overnight at 105°C, cooling them in a desiccator, and weighing. Samples were then heated in a muffle furnace for 3 hrs at 600°C, cooled in a desiccator, and reweighed (Booth 1971, Panikkar and Rajan 1970). This loss-on-ignition method may overestimate the available carbon due to volatilization of car-

bonates (Ball 1964, Davies 1974). The pH was measured by a Beckman electromate pH meter. A slurry of sand mixed with distilled water (1:1 w/w) was stirred by a magnetic stirrer during measurement. Sand grain size distribution was determined by sifting oven dried sand through a USDA sieve set (Humboldt Mfg. Co., Norridge, Ill.). Percent carbonate was determined by weighing residue left after treating sand with an excess of concentrated HCl.

Media

The media for isolation of the heterotrophic microbes were (1) Fuller's modified Vishniac agar (Fuller, Fowles, and McLaughlin 1964), (2) V-8 juice agar (American Type Culture Collection *Catalogue of Strains*, Ed. 8, 1968), (3) sodium caseinate agar (BBL 11626), (4) TGY (American Type Culture Collection *Catalogue of Strains*, Ed. 8, 1968), and (5) Kuster's starch agar (Kuster and Williams 1964). Media were supplemented to inhibit or enhance growth of a given group of microorganisms. To inhibit bacteria, we used 100 units of penicillin G (Eli Lilly and Co.) and 100 units streptomycin sulfate (Eli Lilly and Co.) per ml. To inhibit fungi, we used mycostatin (E. R. Squibb and Sons, Inc.) at the rate of 100 units per ml, and to select for phycomycetes and some dematiaceous fungi (Edgington, Khew, and Barron 1971), benomyl (Benlate, E. I. DuPont deNemours and Co.) was autoclaved separately in distilled water and added as 0.1 g per 1000 ml.

Incubation

Plates were incubated at room temperature (21–23°C), 50 cm from two 40-watt fluorescent lights. Duplicate sets were incubated in candle jars (Emerson and Held 1969) for microaerophilic species. Beakers of saturated sodium chloride were placed in the candle jars to control condensation (Chen and Griffin 1966). Standard references were used for identifications.

Isolates selected for physiological testing were in culture less than 3 months to limit adaptation to in vitro conditions (ZoBell and Michener 1938). Small cubes of mycelium from a plate of Emerson YpSs Agar (Difco 0739) made with seawater (35 o/oo salinity) were used for inoculum. Salinity tests were conducted on Emerson YpSs Agar with salinities of 0, 7, 14, 21, 28, 35, 42, 56, and 70 o/oo made either by concentration of 35 o/oo salinity seawater or dilution with distilled water. Three petri plates were set up for each salinity test. Plates were incubated at 25°C in the dark. Each plate was measured for radial growth at four points.

To test for pH preferences, we used an Emerson YpSs medium, made from ingredients as a broth, dispensed at 50 ml per 125 ml flask and incubated on a rotary shaker at 125 r/min or, in the case of *Alternaria alternata* (Fr.) Keissler, at 100 ml per 250 ml flask, and incubated on a rotary shaker at 250 r/min. The pH of the medium was adjusted with 0.1N KOH and 0.1N HCl. Inoculation of each flask was from 0.1 ml of spores suspended in demineralized water. Three replicates were set up for each pH. Flasks were incubated at 23°C. After 2 days, microbial mass was measured by filtering onto tared filter paper, oven drying at 75°C for 2 days, cooling in a desiccator, and weighting.

Index of Similarity

Fungal populations from each zone were compared on the basis of presence or absence of species by the Dice similarity quotient (SQ) (Cheetham and Hazel 1969), which provides an index of similarity for species of any two samples:

$$SQ = \frac{2C}{n_1 + n_2} \times 100,$$

where C = the number of species in common between two samples; n_1 = the number of species from site 1; and n_2 = the number of species from site 2. With perfect similarity, the index would equal 100 percent. SQ values arranged into matrices and clustered by the weighted pair group method (Sokal and Sneath 1963) were used to determine differences in fungal populations.

TABLE 1

HETEROTROPHIC MICRO-ORGANISMS FROM SELECTIVE ISOLATION MEDIUM YIELDING HIGHEST NUMBER PER GRAM OF SAND AND ORGANIC CONTENT OF SAND

BEACH AND ZONE	BACTERIA	ACTINOMYCETES	FUNGI	ORGANIC CONTENT (%)
Kahala Beach, Oahu				
Land	300,000	24,000	11,000	—
Supratidal	243,000	16,000	9,800	4.7 ± 0.49
Intertidal	6,700	0	7	4.6 ± 0.55
Subtidal	5,700	33	210	7.2 ± 0.40
Black	30,800	0	3	6.5 ± 0.22
Mahana Bay Beach, Hawaii				
Supratidal	269,000	15,000	15,900	0.7 ± 0.12
Intertidal	52,000	0	2	1.0 ± 0.05
Subtidal	230	0	1	2.0 ± 0.50
Punaluu Beach, Hawaii				
Supratidal	4,300	30	80	0.4 ± 0.10
Intertidal	13,000	0	2	0.1 ± 0.02
Subtidal	120	0	1	0.2 ± 0.04

NOTE:—no data available.

TABLE 2

CHEMICAL AND PHYSICAL CHARACTERISTICS OF THREE HAWAIIAN BEACHES

	ISLAND OF HAWAII		ISLAND OF OAHU
	MAHANA BAY BEACH	PUNALUU BEACH	KAHALA BEACH
Sand temperature*	32 C	51 C	30 C
pH (slurry)†	8.6	7.1	8.8
Salinity (o/oo)†	34.8	23.8	25.0
Carbonate content	6.3% ± 0.31‡	2.9% ± 0.35	99.3% ± 0.04

*Measured at midday under bright sun conditions.
†At low tide.
‡Standard error.

RESULTS

Organic content, of prime importance for heterotrophic existence, varied markedly among the three beaches, but was fairly consistent throughout a given beach (Table 1). It was not correlated with numbers of micro-organisms in any beach zone. Punaluu Beach and Mahana Bay Beach, the volcanic beaches, were uniformly low in organic content (0.1 to 2.0 percent); Kahala carbonate beach had a higher organic content (4.6 to 7.2 percent). More micro-organisms were isolated from beaches containing high quantities of organic matter than from beaches low in organic matter.

Physical Characteristics of Beaches

Carbonate content of sand from the three beaches differed significantly (Table 2). It was 3 percent at Punaluu Beach, 6 percent at Mahana Bay Beach, and 99 percent at Kahala Beach. The lower percentages reflect the two beaches' volcanic origin while the higher percentage on Kahala Beach reflects

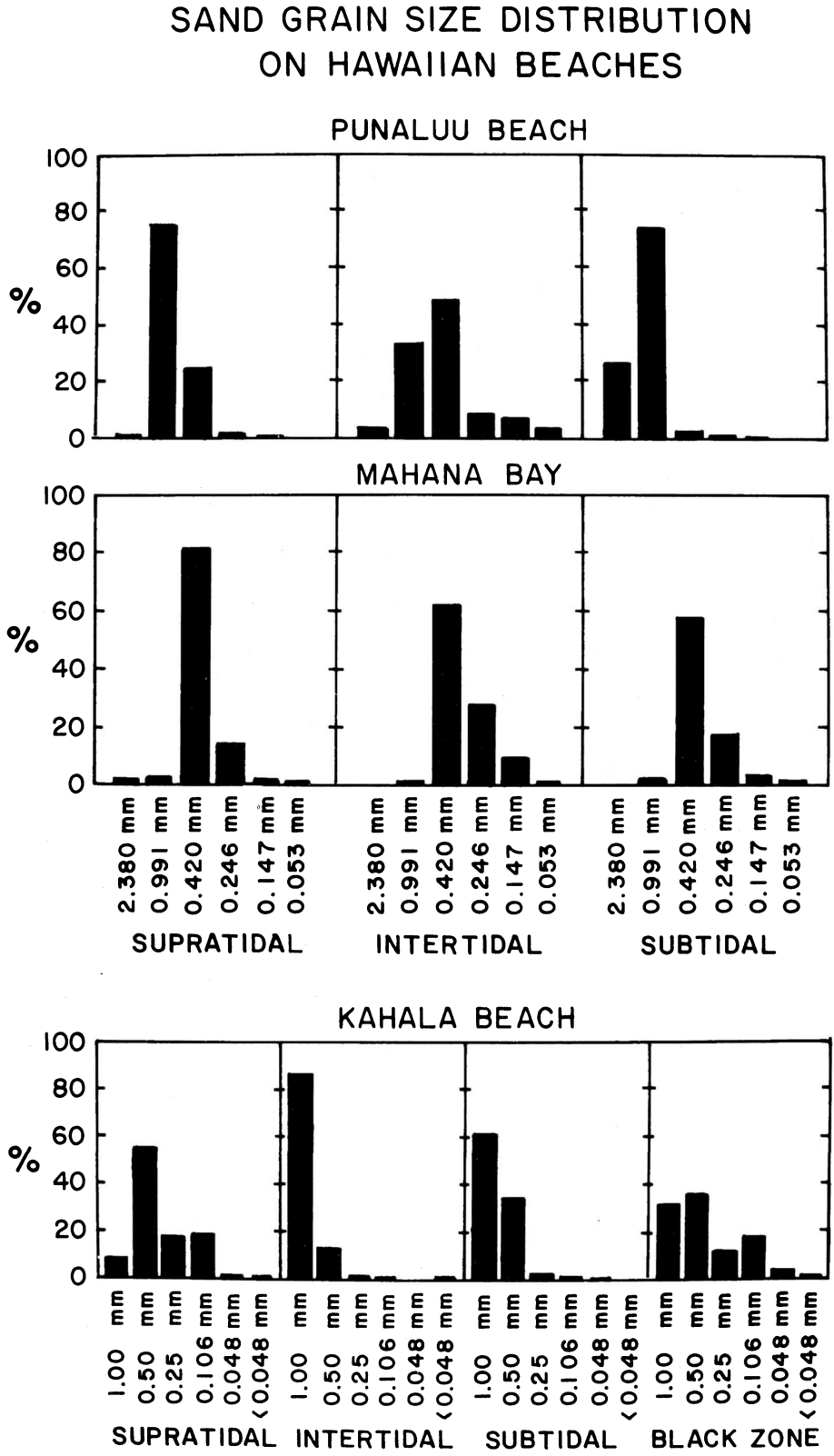

FIGURE 2. The size of sand grains differs among the three beaches studied: Punaluu Beach and Mahana Bay Beach, Island of Hawaii, and Kahala Beach, Island of Oahu. Quantities of more than 10 percent fine grain sand (0.250–0.125 mm) are typical of black zones.

TABLE 3

SIMILARITY QUOTIENTS (SQ) BETWEEN BEACHES

NUMBER OF SPECIES*			
Mahana Bay Beach, Hawaii	Punaluu Beach, Hawaii	Common	SQ(%)
40	49	25	56
Mahana and Punaluu	Kahala, Oahu (without L and B)†		
63	64	26	41
Kahala Beach, Oahu	Enewetak, Marshall Islands		
73	116	46	48
All Hawaiian beaches	Enewetak, Marshall Islands		
107	116	48	43

* Excluding the Sphaeropsidales which are not taxonomically comparable.
† L = land zone, B = black zone.

the activity of foraminifera with shells of calcium carbonate.

Punaluu Beach had an extremely coarse sand. Of the sand in the subtidal, 98 percent was larger than 1 mm, and 25 percent exceeded 2.4 mm (Figure 2). Mahana Bay Beach had small sand grains. Of the sand in the supratidal zone, 80 percent was between 0.4 and 1.0 mm in diameter (Figure 2). In the intertidal and subtidal, there was more sand in the 0.4- to 1.0-mm class than in other classes. Pieces larger than 1.0 mm were rare. Kahala Beach, unlike the other beaches which are constantly mixed throughout by wave action, showed considerable sorting of sand forming distinct zones (Figure 2). Fragments of more than 1.0 mm constituted more than 80 percent of the intertidal sand, and more than 60 percent of the subtidal sand. The black zone is rarely mixed by wave action due to the concentration of fine sands. This restricted circulation of oxygenated water is favorable for development of a black anaerobic zone (Brafield 1965).

Salinity of interstitial water on the beach was a constant 35 o/oo at Kahala Beach and Mahana Bay Beach. It measured 24 o/oo at Punaluu Beach due to groundwater seepage.

The three beaches differed markedly in temperature. At Punaluu Beach a high temperature of 51°C was measured 5 mm beneath the surface at midday. The upper layers of the black sand at Punaluu Beach dried after each tide. Because both Mahana Bay Beach and Kahala Beach are lighter colored, they probably reflect much of the incoming radiation which keeps the sand cooler. With maximum temperatures below 40°C, moisture retention would increase.

Mahana Bay Beach and Punaluu Beach are volcanic beaches, but Mahana Bay Beach is an alkaline olivine sand with a slurry pH of 8.6. The less alkaline basalt of Punaluu Beach had a pH of 7.1. Kahala Beach with its carbonate buffer system had a pH of 8.8.

Heterotrophic Micro-organisms

The total number of heterotrophic micro-organisms was low, ranging from 120 to 300,000 per g wet sand. The number of organisms decreased as the time of submergence was increased from the supratidal to subtidal (Table 1). The number of heterotrophic micro-organisms does not necessarily correlate with organic content (Table 1). The number of micro-organisms was unrelated to the organic content of each zone. The organic content was fairly consistent throughout the beach, while the number of micro-organisms decreased as the time submerged increased (Table 1).

Measures of Zonation

When the alkaline olivine sand beach at Mahana Bay Beach was compared with the acidic basalt sand at Punaluu Beach, the similarity quotient was 56 percent. When the two volcanic beaches were compared with the Kahala carbonate beach, the SQ was 41 percent (Table 3).

When the fungal populations of the beach zones at Kahala Beach were compared with

TABLE 4

SIMILARITY QUOTIENTS (SQ) FOR ZONES*

NUMBERS OF SPECIES											
KAHALA BEACH, OAHU				MAHANA BAY BEACH, HAWAII				PUNALUU BEACH, HAWAII			
L	Sp	Common	SQ(%)	Sp	I	Common	SQ(%)	Sp	I	Common	SQ(%)
24	20	11	50	23	33	18	64	24	31	13	47
L and Sp	I			I	Sb			I	Sb		
33	21	12	44	33	21	19	70	31	31	20	65
L and Sp	Sb and B			Sp	Sb			Sp	Sb		
33	63	26	53	23	21	12	55	24	31	16	58
I	Sb and B										
21	63	18	43								
L	Sb and B										
24	63	17	39								
Sb	B										
58	21	16	41								
Sb (anaerobic)†	B(anaerobic)										
41	13	9	33								
B(aerobic)†	B(anaerobic)										
13	13	5	39								

*L = land zone; Sp = supratidal; I = intertidal; Sb = subtidal; B = black zone.
†Anaerobic and aerobic are incubation conditions.

TABLE 5

SPECIES OCCURRING IN ONLY ONE ZONE AND SPECIES OCCURRING IN ALL ZONES

	HAWAII		OAHU
	MAHANA BAY BEACH	PUNALUU BEACH	KAHALA BEACH
One species			
Land			5/24
Supratidal	5/23	7/24	1/20
Intertidal	8/33	10/31	3/21
Subtidal	2/21	7/31	23/58
Black zone			3/21
All species			
Without land or black zone	12/40	12/49	10/64
Without land			4/69
Without black zone			6/70
With land and black zone			3/74

each other (Table 4), most zones had about a 50 percent SQ. About one-half of the fungi were distributed throughout the beach, and about one-half were restricted to one or a few zones. Mahana Bay Beach also had a high degree of similarity of fungal populations among its zones—55 to 70 percent (Table 4). Punaluu Beach showed a similar pattern—47 to 65 percent (Table 4).

Another measure of the uniqueness of the fungal populations in any zone is the number of species found exclusively in one zone (Table 5). On volcanic beaches with wave mixing, and therefore little zonation, the number generally ranged from 10 to 30 percent of each zone's species. At Kahala Beach, the percent of species specific to each zone was low (less than 20), except for the subtidal reef flat (40).

The contrasting measure of zonation is to compare the number of species common to all zones in a beach (Table 5). About 25 percent of the fungal species isolated from each of the

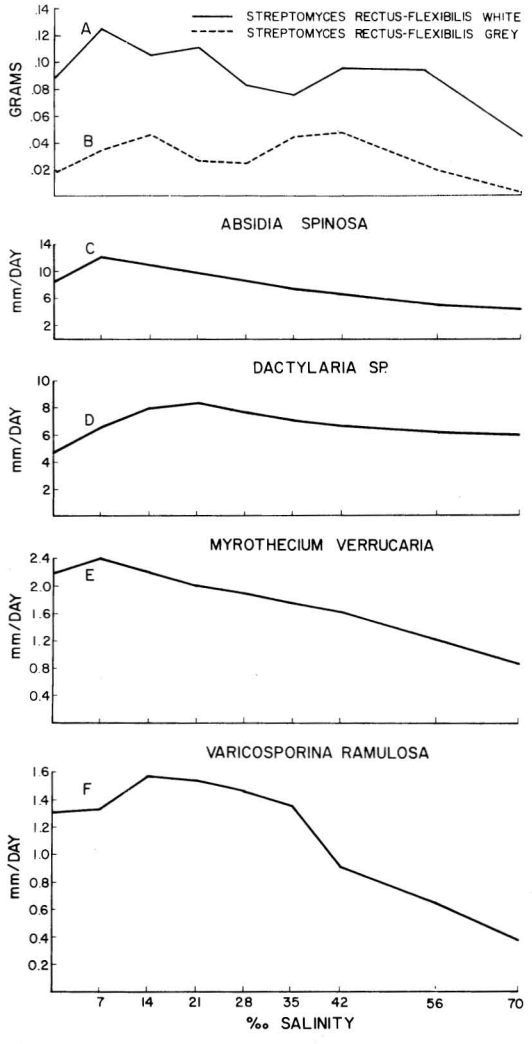

FIGURE 3. Growth response to salinity of actinomycetes from the supratidal zone, Mahana Bay Beach, Hawaii. (A) *Streptomyces* rectus-flexibilis white, and (B) *Streptomyces* rectus-flexibilis grey. Three fungi from the subtidal zone: (C) *Absidia spinosa*, S-1121, Mahana Bay Beach, (D) *Dactylaria* sp., S-1381, Punaluu Beach, (E) *Myrothecium vessucaria*, S-1646 from Kahala Beach; and one fungus from the supratidal, and (F) *Varicosporina ramulosa*, S-1278 from Punaluu Beach.

volcanic beaches were found in all zones. The volcanic beaches also showed little zonation by SQs (Table 3). When the same zones were considered on Kahala Beach, which had more zonation, fewer (16 percent) were common to all zones.

Physiological Adaptations

Actinomycetes were virtually absent in the intertidal and subtidal zones (Table 1). Two species of *Streptomyces*, isolated from Hawaiian beaches, reflected a general preference for low salinity, but had a sufficiently wide tolerance so that salinity alone was unlikely to account for their absence from the subtidal zone (Figures 3*a*, *b*).

Fungi were always present in the subtidal zone, but were generally few in number (Table 1). Salinity did not seem to be a controlling factor for the fungi tested (Figures 3*c*–*f*). Although filamentous phycomycetes are rare in tropical marine habitats (Lee and Baker 1972), *Absidia spinosa* Lendner, isolated at Kahala Beach, grew through the normal range of oceanic salinity (Figure 3*c*). Maximum radial growth occurred at 7 o/oo salinity. Even when salinity of seawater was twice the normal level, the fungus still grew at one-half the rate that it grew at 7 o/oo. *Dactylaria* sp., *Myrothecium verrucaria* (Alb. and Schw.) Ditm., and *Varicosporina ramulosa* were common isolates from Punaluu Beach (Table 6) and made up a significant part of the community. *Dactylaria* sp. was the most frequently isolated fungus. *Dactylaria* sp. and *V. ramulosa* were isolated from no other beach (Table 6). The seawater salinity at Punaluu Beach was 24 o/oo (Table 2) and *Dactylaria* sp. and *V. ramulosa* showed maximum growth at that level (Figures 3*d*, *f*). *Myrothecium verrucaria* grew best at 7 o/oo salinity, but could grow at 24 o/oo (Figure 3*e*).

High temperature combined with drying produces a stress which might account for the few organisms at Punaluu Beach (Table 1). Temperature responses of three fungal isolates showed that *Dactylaria* sp. did not grow in vivo daytime beach temperatures (Figure 4). *Myrothecium verrucaria* and *Varicosporina ramulosa* grew best at temperatures at or above 30°C, but could not grow at the 50°C temperatures of the Punaluu Beach supratidal at midday (Figure 4).

Fungi isolates from all beaches were tested for pH response by gravimetric growth measurement in liquid shake culture. Maximum growth for all test isolates occurred in the acid

TABLE 6

FUNGI ISOLATED FROM HAWAIIAN BEACHES AND THEIR SOURCES

TAXA	MAHANA BAY BEACH*			PUNALUU BEACH*			KAHALA BEACH*						
										4		5	
	2	3	4	2	3	4	1	2	3	+	−	+	−
Phycomycetes													
Absidia corymbifera (Chon) Saccardo & Trotter		×											
A. spinosa Lendner			×	×							×		
Cunninghamella bainieri Naumov											×		
Mucor albo-ater Naumov							×				×	×	
Mucor sp.							×		×	×			
Rhizopus nigricans Ehrenberg							×				×		×
Ascomycotina													
Aspergillus variecolor Berkeley & Broome											×		
Chaetomium globosum Kunze ex Fries				×									
C. indicum Corda													×
C. robustum Ames							×	×					
Guignardia? sp.						×							
Gymnoascus reesii Baranetzky												×	×
Leptosphaerulina sp.									×				
Melanospora sp.							×						
Microascus trigonosporus Emmons & Dodge								×	×	×			
Podospora sp.					×								
Pseudosphaeriales, sp. indet.						×							
Fungi Imperfecti													
Sphaeropsidales													
Lasiodiplodia theobromae (Pat.) Gritt & Maubl.											×		
PYC-I	×	×	×	×	×	×	×	×	×	×	×	×	
PYC-III					×					×	×		
PYC-V							×			×	×		
PYC-VII										×			
PYC-VIII						×							
PYC-IX					×								
Moniliales													
Moniliaceae													
Acremonium bacillisporum (Onions & Barron) W. Gams									×		×		
A. strictum W. Gams		×			×	×				×			
A. zeae? W. Gams & Sumner									×	×			

TABLE 6 (*continued*)

TAXA	MAHANA BAY BEACH*			PUNALUU BEACH*			KAHALA BEACH*						
										4		5	
	2	3	4	2	3	4	1	2	3	+	−	+	−
Acremonium sp. (triangular spores)										×			
Acremonium sp.				×									
Aspergillus flavipes (Bain. & Sart.) Thom & Church		×	×										
A. flavus Link	×	×	×	×									
A. ochraceus Wilhelm	×	×		×	×	×				×	×		
A. sclerotiorum Huber	×												
A. terreus Thom		×	×	×	×	×				×	×	×	×
A. tubingensis (Schober) Mosseray		×	×	×	×	×				×	×	×	
A. ustus (Bain.) Thom & Church	×	×		×		×	×	×	×		×		×
A. versicolor (Vuill.) Tiraboschi				×		×	×	×					
Cylindrocephalum sp.		×			×	×							
Diheterospora catenulata Kamyschko					×								
Fusidium griseum Link	×	×	×								×		
Gliocladium fimbriatum Gilman & Abbott										×			
G. roseum (Link) Thom					×	×							
Oidiodendron sp.		×								×	×	×	
Paecilomyces variabilis Barron		×										×	
Paecilomyces sp. (polyverticillate)							×					×	
Penicillium citreoviride Biourge										×			
P. janthinellum Biourge		×			×	×							
P. jenseni Zaleski				×		×			×	×	×	×	×
P. lilacinum Thom										×	×		×
P. miczynskii Zaleski						×							
P. multicolor G.-M. & Poradielova											×		
P. piscarium Westling									×				
Phialomyces sp.										×			
Polyscytalum pustulans type										×			
Trichoderma viride Pers. ex S. F. Gray	×	×	×	×	×	×	×	×		×	×		
Varicosporina ramulosa Meyers & Kohlm.					×	×							
Verticillium lecanii (Zimm.) Viegas										×			
V. psalliotae Treschow											×		
V. tenuissimum Corda							×		×	×	×		×
Verticillium sp.		×	×										
Yeast					×						×		

TABLE 6 (*continued*)

TAXA	MAHANA BAY BEACH*			PUNALUU BEACH*			KAHALA BEACH*						
										4		5	
	2	3	4	2	3	4	1	2	3	+	−	+	−
Dematiaceae													
Alternaria alternata (Fr.) Keissler	×	×	×	×	×	×	×	×		×	×		×
Aureobasidium pullulans (DeBary) Arnaud													
Bipolaris australiensis (M. B. Ellis) Tsuda & Ueyama	×	×	×	×	×	×	×	×	×	×	×		
B. hawaiiensis (M. B. Ellis) Uchida & Aragaki	×	×			×	×		×			×		
Bipolaris sp.					×	×				×	×		
Cladosporium cladosporioides (Fresen.) de Vries	×	×	×		×	×		×	×	×	×	×	
C. oxysporum Berk. & Curt.	×	×		×	×	×	×	×	×	×	×		
Clavariopsis bulbosa Anastasiou								×					
Curvularia clavata Jain								×					
C. intermedia Boedijn					×								
C. lunata (Wakker) Boedijn							×	×		×	×	×	
Curvularia sp.		×											
Dactylaria sp.				×	×	×							
Exserohilum rostratum (Drechsl.) Leonard & Suggs	×			×	×			×		×	×		
Humicola fuscoatra Traaen									×	×		×	×
H. grisea Traaen	×	×	×	×									
Memnoniella echinata (Riv.) Galloway										×			
Nigrospora state of *Khuskia oryzae* Hudson	×	×	×	×									
Periconia sp.		×											
Phialophora verrucosa Medlar.										×	×		
Pithomyces atro-olivaceus (Cooke & Harkness) M. B. Ellis						×	×			×	×		
Rhinocladiella cellaris (Pers. ex S. F. Gray) M. B. Ellis								×		×			
Scolecobasidium humicola Barron & Busch	×	×	×					×		×			
S. variabile Barron & Busch							×						

TABLE 6 (*continued*)

TAXA	MAHANA BAY BEACH*			PUNALUU BEACH*			KAHALA BEACH*						
										4		5	
	2	3	4	2	3	4	1	2	3	+	−	+	−
Scopulariopsis brevicaulis (Sacc.) Bainier								×	×	×	×		
S. brumptii Salvanet-Duval	×	×	×	×			×						
Stachybotrys atra Corda							×	×	×	×			
Stachybotrys state of *Melanopsamma pomiformis* (per. ex Fr.) Sacc.	×	×											
Stemphylium state of *Pleospora herbarum* (Pers. ex Fr.) Rabenh.	×			×	×	×							
S. vesicarium (Wallr.) Simmons	×												
Ulocladium alternariae (Cke.) Simmons						×							
U. consortiale (Thum.) Simmons		×	×										
Stilbaceae													
Graphium penicillioides Corda								×	×		×		
G. putredinis (Corda) Hughes													×
Tuberculariaceae													
Cylindrocarpon sp.									×				
Epicoccum purpurascens Ehrenb. ex Schlecht.					×		×						
Fusarium lateritium†					×								
F. moniliforme	×	×		×		×				×	×		
F. roseum	×												
F. solani	×	×	×	×	×	×	×	×	×	×	×		×
F. stoveri?			×										
Myrothecium brachysporum Nicot							×						
M. roridum Tode ex Fr.						×	×			×	×		
M. verrucaria (Alb. & Schw.) Ditm. ex Fr.											×		
Mycelia Sterilia													
Papulospora sp.										×			
Nonsporulating Mycelium													
Moniliaceae		×	×	×	×	×			×	×	×		
Dematiaceae		×	×		×	×			×	×	×	×	×

*1 = land zone, 2 = supratidal zone, 3 = intertidal zone, 4 = subtidal zone, 5 = black zone, + = aerobic, − = anaerobic.
† *Fusarium* species after Snyder and Hansen system.

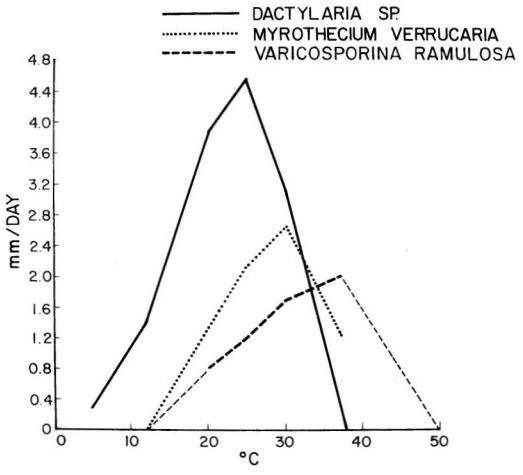

FIGURE 4. Growth responses to temperature of *Dactylaria* sp., S-1381, *Myrothecium verrucaria*, S-1646, and *Varicosporina ramulosa*, S-1278. The broken lighter lines for *Varicosporina ramulosa* indicate probable slope to the point of no growth.

range despite the neutral to alkaline conditions of the beach habitats (Figure 5).

DISCUSSION

The total number of heterotrophic microorganisms on Hawaiian beaches was low. The number of organisms increased as the time of submergence from supratidal to subtidal increased (Table 1). This trend has been noted for atoll beaches (Dunn and Baker 1983). Time submerged seemed to be a major determinant of the number of microbes in the zones.

Salinity did not seem to be the governing factor for the presence or absence of fungi on the beaches (Figures 3*c–f*). Some of the isolates were adapted to the salinity of the beach where isolated while others had lower optima. All species tested grew better at salinity levels above 0 o/oo and can grow at any salinity level normally found in beaches of the Hawaiian Islands. High salinities, due to evaporation in beach sands, may have caused selection for some species of fungi which tolerate a wide range of salinities or which grow better in the presence of high salinities (Figure 3). Salinity optima are probably just one factor in controlling distribution with zones of a beach.

High temperature combined with drying produces a stress condition which might account for the fewer micro-organisms on the hotter Punaluu Beach than on the two cooler beaches (Table 1). Three fungal isolates tested from Punaluu, including *Varicosporina ramulosa*, did not grow at normal in vivo daytime beach temperatures (Figure 4). This response suggests that the fungi only tolerate the high temperatures at midday and perhaps grow only when temperatures are lower (Boyd and Kohlmeyer 1982). Temperature could help reduce the number of viable propagules in the supratidal. Resistance to desiccation and long periods of high temperature would be essential for survival of propagules. Sclerocarps of *V. ramulosa* can survive extreme temperatures of 45°C and 70°C (Kohlmeyer and Charles 1981). Temperature has no effect on zonation of microbes in areas of frequent wave action.

The fungi have not adapted to the pH of the seawater and the beaches, which are neutral to alkaline. They all showed optima in the acidic range which is typical for fungi, although two of the eight tested had bimodal optima with secondary peaks in the alkaline range.

All three beaches shared several species, but each beach also had species unique to it. The Kahala carbonate beach had a higher similarity (48 percent) than did the two volcanic beaches (41 percent). In comparing zones within each beach, we found that Mahana Bay Beach had a high degree of similarity of fungal populations among its zones (55 to 70 percent). Punaluu Beach showed a similar pattern (47 to 65 percent). These two reefless beaches have steep slopes, with large waves sweeping into shore and shifting sand around the beach. This process makes zonation down the beach difficult. The zones of the reef-protected Kahala Beach had slightly less similarity (50 percent), reflecting the higher stability.

Few of the SQs were large enough or small enough to allow strong statements about the comparisons between beaches or zones of the beaches. Other evidence, such as occurrence of unique species, also needs to be considered. This measure shows that the Kahala reef flat is

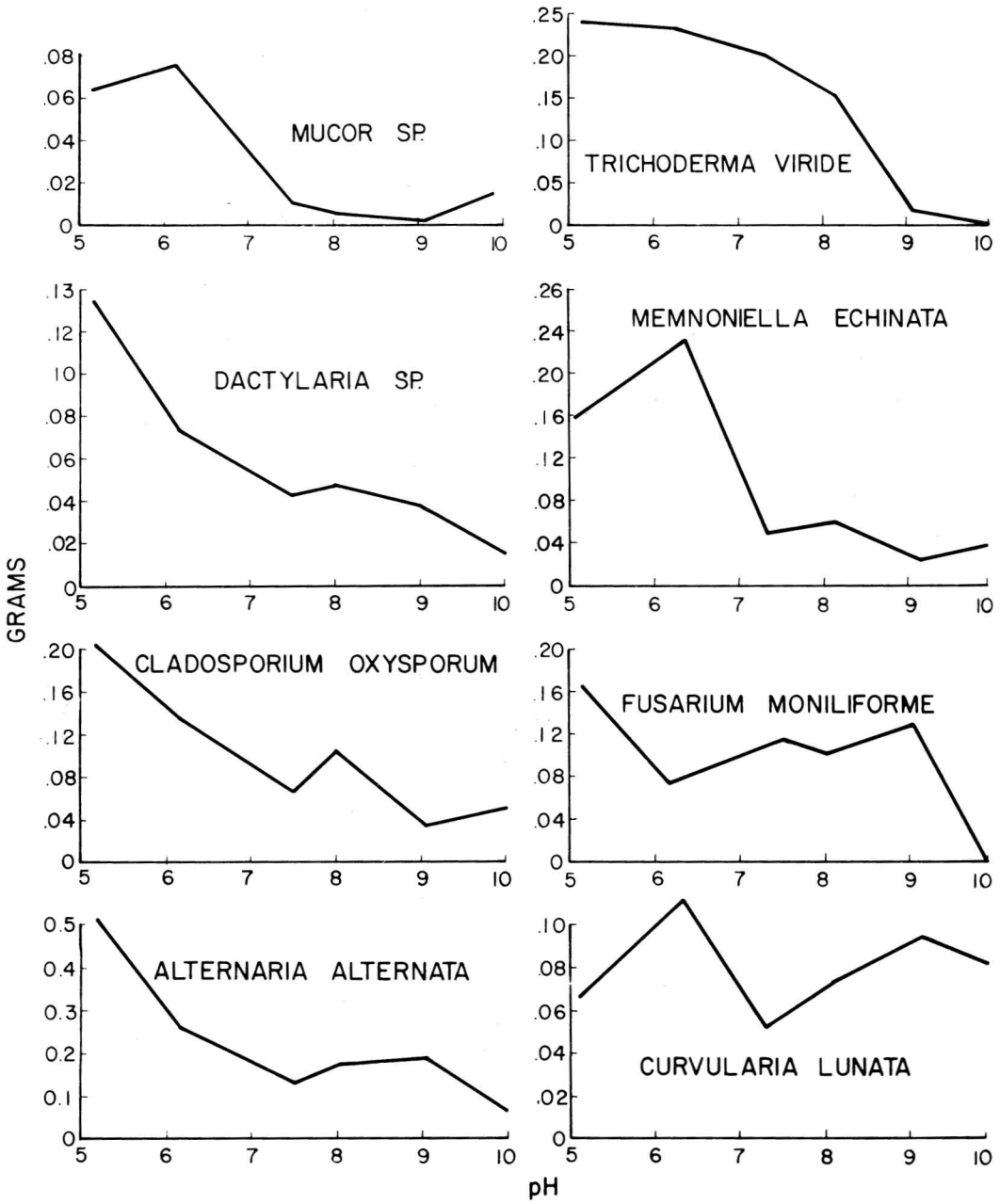

FIGURE 5. Growth response to pH of *Mucor* sp., S-1121, Mahana Bay Beach subtidal zone; *Dactylaria* sp., S-1381, Punaluu Beach subtidal zone; *Cladosporium oxysporum*, S-1300, Punaluu Beach subtidal zone; *Alternaria alternata*, S-1120, Mahana Bay Beach subtidal zone; *Trichoderma viride*, S-1651, Kahala Beach subtidal zone; *Memnoniella echinata*, S-1635, Kahala Beach subtidal zone; *Fusarium moniliforme*, S-1184, Punaluu Beach supratidal zone; and *Curvularia lunata*, S-1651, Kahala Beach subtidal zone.

TABLE 7

DISTRIBUTION OF FUNGAL GROUPS IN HAWAIIAN BEACHES, BY NUMBER OF SPECIES*

	KAHALA BEACH, OAHU						MAHANA BAY BEACH, HAWAII				PUNALUU BEACH, HAWAII			
	Total	La	Sp	I	Sb	B	Total	Sp	I	Sb	Total	Sp	I	Sb
Phycomycetes	5	3	0	1	5	2	2	0	1	1	1	1	0	0
Ascomycetes	7	2	2	2	2	2	0	0	0	0	4	1	1	2
Basidiomycetes	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fungi Imperfecti														
Sphaeropsidales	5	2	1	1	5	1	1	1	1	1	4	1	3	2
Melanconiales	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Moniliales														
Moniliaceae	26	5	3	6	22	9	15	6	14	7	17	9	11	13
Dematiaceae	19	8	12	6	16	4	16	13	13	8	16	9	11	9
Stilbaceae	2	0	1	1	1	1	0	0	0	0	0	0	0	0
Tuberculariaceae	7	4	2	3	4	2	4	3	2	2	5	2	3	3
Mycelia Sterilia	1	0	0	0	1	0	0	0	0	0	0	0	0	0
Nonsporulating mycelium	2	0	0	2	2	1	2	0	2	2	2	1	2	2
Total no. of species	74	24	21	22	58	22	40	23	33	21	49	24	31	31

*La = land; Sp = supratidal; I = intertidal; Sb = subtidal; B = black zone.

the only area with many unique species as might be predicted because it is a stable area. In contrast, the volcanic beaches have the most species found in all zones.

Several fungi were notable for their occurrence patterns on the three beaches studied (Table 6). Typically, the filamentous Phycomycetes are not common in tropical marine areas (Table 7) (Sparrow 1948, Steele 1967, Lee and Baker 1972). There were few isolated. One chytrid, *Rhizophlyctis rosea* (De Bary and Waronin) Fischer, has been reported from above the high tide line of Punaluu Beach on the Island of Hawaii (Sparrow 1965). Ascomycetes were rare on the volcanic beaches, but were more common on the Kahala carbonate beach (Table 7). *Gymnoascus reesii* Baranetzky and *Microascus trignosporus* Emmons & Dodge were isolated from Kahala Beach. *Lasiodiplodia theobromae* (Pat.) Gritt & Maubl., a member of the Sphaeropsidales, was common at Kahala Beach and is known from the Central Pacific (Dunn and Baker 1983).

In the Hawaiian Islands the great majority of fungal species detected were members of the Moniliales with species about evenly divided numerically between Dematiaceae and Moniliaceae. *Acremonium bacillisporum* (Onions & Barron) W. Gamms was a predominant member of the carbonate sand community. The Aspergilli constituted a major portion of the isolates but showed little zonation or specificity for any beach (Table 6). The Penicillia were important members of the fungal populations of the beaches in the Hawaiian Islands. *Varicosporina ramulosa* Meyers and Kohlm. was common on Punaluu Beach. *Varicosporina ramulosa* is a marine fungus reported as typical of the tropical and subtropics but also found in the warm temperate zones during the summer (Boyd and Kohlmeyer 1982). *Alternaria alternata* (Fr.) Keissler was a frequent isolate from Hawaiian beaches. Both *Biploraris australiensis* (M. B. Ellis) Tsuda & Ueyama and *B. hawaiiensis* (M. B. Ellis) Uchida & Aragaki were frequent on all beaches. *Graphium penicillioides* Corda and *G. putredinis* (Corda) Hughes were found at the Kahala carbonate beach; *G. putredinis* was found frequently on plates from black zone sands incubated in candle jars. *Fusarium solani* (sensu Snyder & Hansen) was a common isolate at Kahala Beach.

A parallel study was done on the filamentous fungi of the carbonate beaches of Enewetak Atoll (Dunn and Baker 1983). The filamentous Phycomycetes were less common than in Hawaii, possibly because of the lack of reintroduction to the beach in land runoff.

Many of the Ascomycetes from Enewetak carbonate beaches were the same species found on the carbonate Kahala Beach. Although the species were the same, the predominance was different. The volcanic beaches had few similarities. Both Enewetak and Hawaii showed the same predominance of Moniliales with a notable pattern. The Penicillia, which are considered common temperate species, were more common in Hawaii than at Enewetak.

Although many species of higher fungi have been isolated from the Hawaiian beaches (Table 6), their activity in the beaches has not been established. Isolations were done with nutrient enrichment plating techniques which only established that viable propagules are present. A standard long-term preservation method for fungal propagules is storage in distilled water (Campbell and Stewart 1980). Low nutrient seawater would act the same for those propagules which can tolerate the salinity (Dunn and Baker 1983). Inhibitors in seawater (Kirk 1980) and in soil (Lockwood 1977) prevent fungal spores from germinating until endogenous or exogenous nutrients change, or exogenous spore inhibitors are removed. These exogenous inhibitors to nonmarine fungi can be partly removed by autoclaving seawater, while most marine fungi can germinate in the untreated seawater. Addition of glucose can also overcome the inhibition (Kirk 1980). One of the few studies of decomposition of subtidal substrate was of *Thalassia testudinum* Konig in the southeastern United States (Newell and Fell 1980, 1982). Newell and Fell showed that nonmarine fungi, although present, played only a minor role in decomposition of the leaves until the leaves were deposited in the intertidal. Then these terrestrial fungi were active. Terrestrial fungi, isolated from the tropical Pacific beaches, may also remain inactive until the toxin-induced dormancy is overcome by close contact with nutrient-rich substrate.

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