THE ISOLATION AND CHARACTERIZATION OF *Thraustochytrium pachydermum* FROM THE SALTON SEA, CALIFORNIA

by

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PREFACE

This study was undertaken as an open-ended pursuit to verify and broaden the knowledge about halophilic Phycomycetes which were believed to inhabit waters of the Salton Sea, California.

The Salton Sea is a body of water which presently exceeds the oceans in salinity per unit volume. Finding of a halophilic fungus or fungi would provide increased data for Mycota obligatory or facultative to saline environments. Phycomycetes are already recognized inhabitants of ocean waters. Studies over the last 12 years have revealed that they also inhabit salt encrusted or saline environments separated from ocean waters.

Thanks are extended to Dr. R. L. Gilbertson, Dr. R. W. Hoshaw and Dr. W. S. Phillips for their guidance and advice in this study.

Thanks are also given to Dr. F. K. Sparrow, Department of Botany, University of Michigan, for his help in verifying the identity of the organism (Thraustochytrium pachydermum Scholz) isolated from the Salton Sea.

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ABSTRACT

The halophilic fungus, Thraustochytrium pachydermum Scholz, was initially isolated from shoreline waters of the Salton Sea, California, on October 19, 1969. Studies reveal its morphology under salinities of ca. 40 o/oo is very close to the form originally isolated from salt encrusted soils by Scholz in 1958.

The organism exhibits both sessile and free living forms when grown in liquid culture. Rhizoids are capable of attachment to glass surfaces. Growth occurred in salinities ranging from ca. 2.5 o/oo to ca. 60 o/oo Salton Sea water. Growth did not occur in salt-free media. As individual carbon sources, sodium acetate, glycerol, and glucose supported growth. Growth took place on pine pollen (Pinus sp.), date palm pollen (Phoenix dactylifera L.), insect exuviae and onion tissue. Human hair failed to support growth. Diplanetism is not present and sexual phases were not recorded. The organism was isolated from ten points around the entire shoreline of the Salton Sea.

INTRODUCTION

Nearly 100 years of work in the isolation of parasitic and saprophytic marine Phycomycetes has resulted in identification of some 50 species. Major studies in marine mycology have taken place within the last 40 years.

A study of parasitism on eel grass conducted in 1933 revealed the destructive nature of Labyrinthula spp., a member of the Labyrinthulales, an order of uncertain affinity (Renn, 1934). Later studies revealed parasitic invasion by Phycomycetes of a number of marine organisms, some of which bore economic importance. Parasitism included infection of the egg mass of the blue crab (Callinectes sapidus) by Lagenidium callinectes Couch; destruction of body musculature, interference with circulation and degeneration of the central nervous system in teleosts by Ichthyosporidium hoferi Plehn and Mulsow; and parasitism of oyster tissue by Dermocystidium marinum Mackin, Owen and Collier.

Phycomycetes also are known to parasitize marine plankton. Their hosts include diatoms (Johnson, 1966), copepods and rotifers (Johnson and Sparrow, 1961).

Present information indicates that marine Phycomycetes occur with less frequency and numbers than freshwater forms (Vishniac, 1955). However, data relative to numbers, frequency, ecology, distribution, trophic activity and parasitism is too limited to establish a profile at the present time.

Recent studies of fungi parasitic on plankton in lakes provide evidence that planktonic blooms can be effected to the extent that the total bound energy in a complete food chain may be reduced (Sparrow, 1968a). Because most studies concerning marine parasitism in plankton are limited and have been conducted near shore, it becomes obvious that fungi may be present in the marine environment to a greater degree than presently believed. The problems in verifying a hypothesis of this kind lie in the vastness of the habitat and limited means of gathering sufficient data.

Among saprophytic marine Phycomycetes, species of Thraustochytrium (Saprolegniales) are distributed world-wide (Sparrow, 1936; Johnson, 1957; Kobayashi and Ookubo, 1953). The preceding investigators have isolated the organism from waters of Europe, Japan and the United States. Its range includes both open waters and bottom sediments.

While marine mycology is not a new discipline, it has not received intensive and coordinated investigation. Until the 1950's most work centered on Ascomycetes and Deuteromycetes. Since that time, detection of increased numbers of both parasitic and saprophytic Phycomycetes has become possible through successful baiting, use of bacterial suppressants (antibiotics), separation of higher fungi from cultures, use of vitamin and protein enriched substrates, and broader knowledge that Phycomycetes frequently require initial incubation of 3 to 5 weeks before detection is possible. In most cases, complete life cycles are not known, zoospores being the commonly recorded reproductive stage.

Most studies have centered on nutritional requirements and tolerance to salinity. Neither of the preceding are conclusive in defining an ecological group. Recent work (Siegenthaler, Belsky and Goldstein, 1967) has shown that Thraustochytrium roseum Goldstein displays specific sodium requirements which appear essential to metabolism. Should further investigations within the genus support this finding, a clear metabolic dependency could be linked to a salt requirement.

Because Mycota other than Phycomycetes are present in waters at or near 34 o/oo salinity, several "working" criteria have been suggested which would better categorize marine fungi. They include: (a) fungi which grow and reproduce in sea water only; (b) those which mostly frequent marine waters; or (c) forms bearing flagellated reproductive stages which may be found in sea water. While no clear delineation exists for marine grouping, it is noted that most Phycomycetes isolated from marine waters appear to be stenohaline (Vishniac, 1955).

Baiting affords the mycologist greatest success in initially "drawing" Phycomycetes from the aquatic environment. Studies may be conducted in bait cultures directly, or the organism may be transferred and propagated on defined media. Baits commonly used are: snake moult, defatted hair, pollen, onion tissue, rose hips, insect exuviae, algae, wood, or bottom-muds (Sparrow, 1960).

Baiting is initiated by placing a small quantity of bait in a covered dish containing water from the habitat. This is incubated in the dark to exclude photosynthesizing organisms. The culture, if effective, is selective for fungi to the general exclusion of bacteria.

Within the Class Phycomycetes, six orders include marine representatives. They are: Chytridiales, Hyphochytridiales, Plasmodiophorales, Saprolegniales, Lagenidiales and Peronosporales (Johnson and Sparrow, 1961).

This study centers on the characterization of Thraustochytrium pachydermum Scholz which was isolated from the Salton Sea, California, on October 19, 1969.

HABITAT DESCRIPTION

The Salton Sea is the largest inland body of water in California. It lies in the Colorado Desert some fifty miles north of the Mexican border (Fig. 1). The average water level is minus 235 feet. The total surface area approximates 340 square miles, and the average depth is some 40 feet (Walker, 1961).

Adjacent shores are generally void of plant life with the exception of the northern and southern ends where mesquite, creosote bush, salt bush, arrow weed and tamarisk may be found in varying abundance. Shorelines are gently sloping, typical of the depression in which the body of water lies.

Waters of the Salton Sea, according to the latest documented work, (Walker, 1961) were reported at 33.68 o/oo salinity, slightly less than that of the oceans (taken at 34 o/oo). More recent values indicate salinity near 40 o/oo accountable to a generally stable surface level, and addition of an estimated 10,000 tons per day of leached salts entering the Salton Sea from irrigation drainage tiles (ca. 2,200 miles) present within the Imperial Valley agricultural complex (Reich, 1969).

No natural inlets empty into the Salton Sea aside from waters of the White Water River which may carry light and very intermittent spring run-off from the San Bernardino Mountains. Both New and Alamo Rivers which drain into the southern end of the Salton Sea are natural channels, but their primary waters are presently agricultural run-off.

A single man-made drainage, the All-American Canal, which enters from the north, carries a regular drainage of irrigation waters from the Coachella Valley. Originating at Yuma, Arizona, the canal carries water through the Coachella Valley with unused portions being terminated in the Salton Sea.

The Colorado Desert experiences some of the highest summer temperatures within the United States. Daytime air temperatures may reach 45°C during latter July and August. Accordingly, water temperatures may reach 31°C from the surface to 10 meters depth. Winter water temperatures approximate 19-20°C from the surface to 10 meters. The twelve month fluctuation is some 11°C for much of the water mass.

Because of its size and shallow depth, currents are limited or non-existent. The temperature gradient does not support an effective thermocline, and tides are of insignificant magnitude to effect water movement. Considerable winds occur in the area due to the proximity of cool, moist coastal air which is separated from the adjacent desert by the westerly Santa Rosa Mountains. Winds created through the low San Gorgonio Pass, to the north, permit entry of coastal air into the hot dry region. As such, water movement is effected by wind action, but winds may be both diurnal and seasonal. Because of poor circulation, anaerobiosis occurs (Walker, 1961) on a seasonal basis at a depth of 12 meters during the summer months of June through August with a return of oxygen to that depth in early spring and late fall (up to 7 ml per liter of oxygen).

Plant life is dominated by species of the Cyanophycophyta, followed by species of the Chlorophycophyta and considerable numbers

of the Pyrrophycophyta and Chrysophycophyta (diatoms).

Invertebrates include the probably dominant barnacle, Balanus amphitrite Darwin, the pile worm, Neanthus succinea Frey and Leuckart, the copepod, Cyclops dimorphus Kiefer, and the rotifer, Brachionus plecatilus Müller.

Teleosts represent the vertebrates with only two being original to the environment, i. e., accountable to the 1905-1907 formation of the present body of water. They are: the desert pupfish, Cyprinodon macuatris Baird and Girard, and the mosquito fish, Gambusia affinis affinis Baird and Girard (de Stanley, 1967).

Beginning in 1950, the California Department of Fish and Game introduced, into the Salton Sea, fish from the upper Gulf of California. Attempts to establish a successful population resulted in over 40,000 fish-plants exceeding 30 different species. At present, bairdiella, Bairdiella icistius Jordan and Gilbert, and the orangemouth corvina, Cynoscion xanthulus Jordan and Gilbert, appear to be in greatest number. Some fish appear to have died while others are present in reduced number based on sampling and those recorded in sportfishing (Walker, 1961).

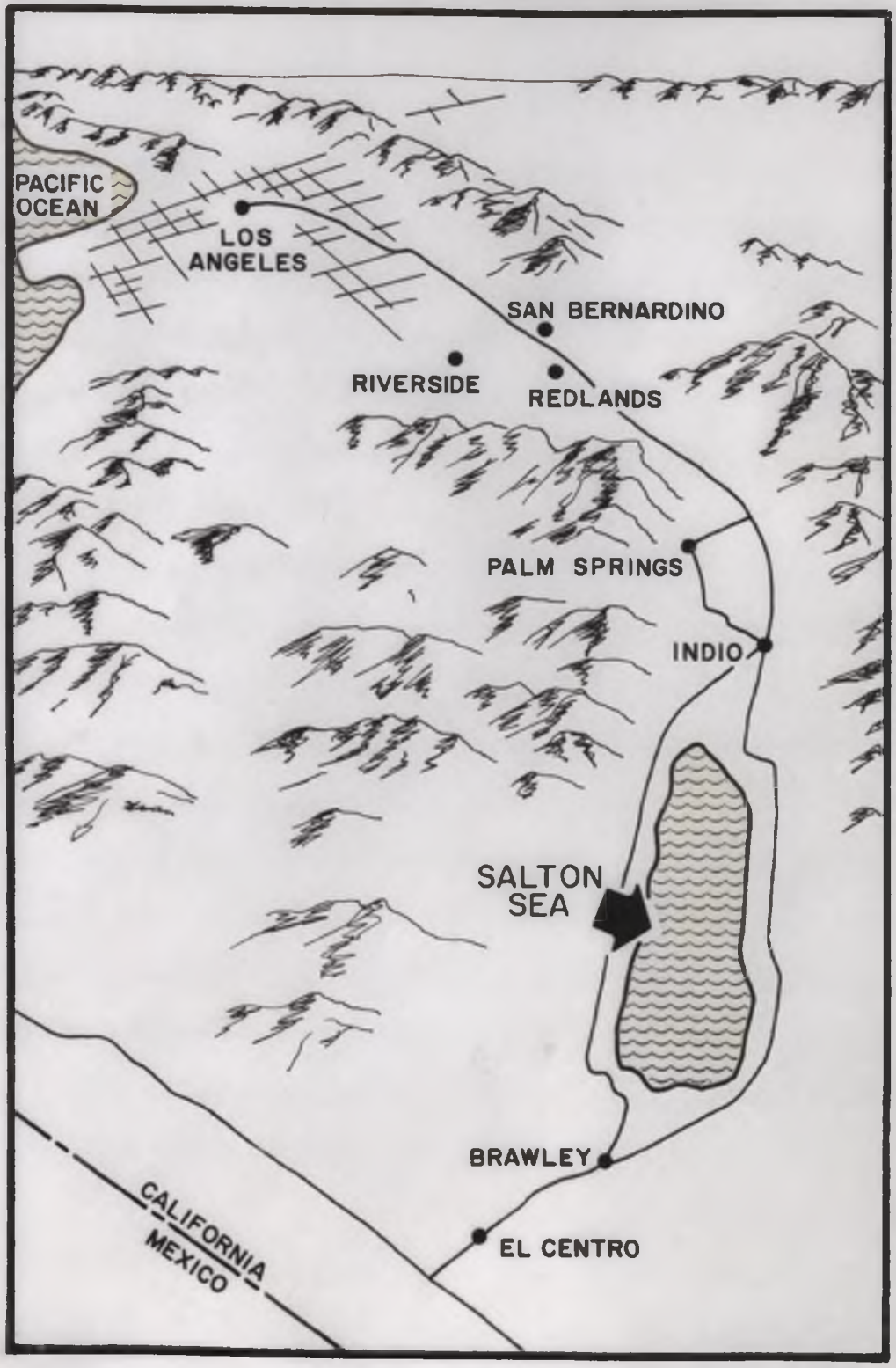

Figure 1. Salton Sea, California location map (adapted from Reich, 1969).

ORIGIN OF THE SALTON SEA

Geologically, the Imperial and Coachella Valleys were formerly covered by an extension of the Gulf of California (Davis, 1907). Evidence of this extension is based on sandstone conglomerate from the Indio mudhills which contain fauna related to gulf forms and distinct from west coast invertebrate assemblages (Bulwalda and Stanton, 1930). Further, Tertiary marine and continental sediments outcrop at various places along the bottom and edges of the Salton Sink.

Isolation of the region appears to be the product of several facts: (a) depression of the present Salton Sea region; (b) uplifting of surrounding mountains; (c) deposition of the Colorado River delta. The river delta is believed to be Quaternary in age being superimposed on a landscape resembling the present one.

That the basin refilled over a span of time was documented by Cohuilla Indians of the region. They indicated the waters of a large lake had disappeared "poco poco" (little by little), but the waters returned and overwhelmed inhabitants, driving them to nearby mountains (Blake, 1854). Returning waters would appear to be a result of wandering movements of the Colorado River rather than a re-entry of the ocean.

Present salinity conditions of both Imperial Valley soils and the Salton Sea are believed to be derived from the Colorado River rather than ocean residue, even though some marine salts were contributed (Bulwalda and Stanton, 1930).

As recently as 1904, the area was dry with brackish pools near the southern end of the present Salton Sea. However, high waters in the Colorado River during the spring of 1905 succeeded in rupturing flood gates of irrigation aqueducts serving agricultural regions within Imperial Valley (Anonymous, 1966). Because the general terrain slopes downward from the region of Yuma, Arizona, into the Salton basin, the entire Colorado River was diverted through Imperial Valley into what was then referred to as the "Salton Sink." Two years were required to regain control of the river and redirect it, culminating in the present body of water.

MATERIALS AND METHODS

Thraustochytrium pachydermum Scholz was baited from a five-week old culture consisting of Salton Sea water, sterile onion tissue and pine pollen. Incubation was conducted in darkness for the first two weeks to exclude photosynthesizing forms. After five weeks, pieces of the onion tissue and pollen grains were aseptically placed on the surface of "Salton Sea" medium consisting of 0.1% glucose, 0.01% yeast extract, 2.0% agar, ½ gram penicillin G, ½ gram streptomycin sulfate and 1 liter of fresh filtered Salton Sea water. The medium was sterilized at 121°C for 15 minutes. After addition of pollen and onion tissue, the petri dish was flooded with sterile Salton Sea water and incubated in darkness for five days. A single drop of the aqueous phase of the preceding culture was removed using a sterile pipet and transferred to new media. Sufficient sterile Salton Sea water to moisten the agar surface was then added. At the end of five days, individual colonies were present and confirmed visually. One of the colonies was removed using a heat sterilized loop and added to "Salton Sea broth" (the aforementioned medium less antibiotics and agar) to establish a pure culture. Stock cultures were maintained at room temperature under normal light conditions.

Determination of species. The organism was initially determined with the aid of Johnson and Sparrow (1961), the species key contained in this thesis, and the original description of T. pachydermum (Scholz, 1958). A description of the isolate and a series of X400

photographs which included vegetative features and sporulation were forwarded to Dr. F. K. Sparrow, University of Michigan, for further confirmation.

Determination of Salton Sea salinity. Salinity was calculated using a specific gravity hydrometer, 1.0000 to 1.0550 (ASTM 111H), manufactured by the Thermometer Corporation of America, Springfield, Ohio; accuracy reproducible to ± 1 o/oo. Shoreline samples were placed in a 2 liter graduated cylinder, on location, and the density and temperature were recorded after a four-minute equilibration period. Ten samples were determined, five along the eastern shoreline and five along the western shore. Recorded densities were corrected to 15°C using a G. M. Manufacturing Company Sea Water Salinity Correction Table.

Utilization of carbon sources. Fifty ml of Salton Sea broth was added to each of three 250 ml flasks. One contained sodium acetate as the carbon source, the second contained glycerol, and the third contained glucose. The control was prepared without addition of a carbon source. Each broth was seeded with 1 ml of 5-day old stock culture and incubated at room temperature in normal light. Growth was determined as a function of visual turbidity, and the presence of active zoospores and thalli which were present beginning on the fourth day of incubation. Ability to utilize a particular carbon substrate was determined to occur where 15 or more zoospores and thalli were present in each of five, X400 microscope fields. The data was recorded as (+) where organisms were present, as previously described, and as (-) where no organisms were present.

Growth as a function of salinity. Seven Salton Sea glucose broths were prepared. The ionic concentration of each was varied by evaporation or dilution as follows: 1.5 concentration, undiluted, 1/2 concentration, 1/4, 1/8, 1/16 and distilled water. Each broth was seeded with 1 ml of 5-day old organisms from a stock culture and permitted to propagate at room temperature and light. Comparative tolerance to varying salinity was determined, beginning on the fourth day. The organism was determined to be "growth tolerant" where 15 or more thalli or zoospores, jointly, were recorded in each of 5 random X400 microscope fields. The data was recorded as (++). The organism was determined to be "withstanding" the growth conditions where 5 or fewer thalli or zoospores, jointly, were recorded in each of 5 random X400 microscope fields. The data was recorded as (+). Growth was determined to be absent where no organisms were recorded in any of 5 random X400 microscope fields. The data was recorded as (-).

Reproducibility of isolation from ten separate points in the Salton Sea. Ten water samples were collected from separate shoreline points (Fig. 2). Each sample was baited, within 12 hours, with pine pollen (Fig. 4) and incubated at room temperature for 10 days. Presence of the organism from any one collection was determined where one or more thalli were present on pollen in a bait culture. Identification was based on thallus morphology.

Determination of growth curve and generation time. The growth curve and generation time were determined by direct cell count. A X400 microscope field was calculated in mm^2 by the formula πr^2. The field constant (k) was determined as:

$\frac{484mm^2}{.120mm^2} = k$, where:

$484mm^2$ = area on the microscope slide to which cells were applied.

$.120mm^2$ = X400 field.

Thus, k = 4,033.

The growth curve was determined as:

$G_c = \log_{10}$ organisms present at any given time.

The growth curve was plotted as a function of organisms present at initiation of growth, after 96, 120, 148, 172 and 196 hours.

The number of generations present from the beginning of incubation to 96 hours was determined as:

$$Gen = \frac{\log A_n - \log A_o}{\log 2}, \text{ where:}$$

A_o = number of organisms present initially.

A_n = number of organisms present after 96 hours.

To begin the determination of generation functions, five, one-liter flasks were prepared with 100 ml of the following medium: 1 gm glucose, 1 gm gelatin hydrolysate, .01 gm (1:20) liver extract, .1 gm yeast extract and 1 liter of filtered Salton Sea water. The medium was sterilized at 121°C for 15 minutes (modified from Fuller, Fowles and McLaughlin, 1964).

The number of thalli per ml of stock culture grown on the aforementioned medium was determined by applying .01 ml of cells to a glass slide. The cells were then covered with a cover slide ($484mm^2$).

Thalli in 25 fields were counted, their average number was then calculated (n); the number of cells present in 1 ml of medium was calculated as:

$$(k)(n)(100) = \text{number of thalli per ml.}$$

After determination of cells per ml of stock culture, 1 ml was aseptically added to each of five growth chambers (replicates) and the number of thalli present per ml at initiation of the generation period was determined as:

$$\frac{\text{cells per ml of stock culture}}{101 \text{ ml}} = \text{cells per ml of incubation medium}$$

Utilization of naturally occurring substrates. Five petri dishes were $\frac{1}{2}$ filled with sterile Salton Sea water. The following sterilized substrates (baits) were added, respectively, to each dish: pine pollen (Pinus sp.), date palm pollen (Phoenix dactylifera L.), onion tissue, defatted human hair and insect exuviae (meal worm). One ml of cells from a 5-day old culture were aseptically seeded into each petri dish. The cultures were permitted to incubate for 7 days at which time visual inspection was conducted to ascertain propagation on the baits. Propagation, or ability to utilize a given substrate was tentatively based on the presence of the organism bound to a substrate.

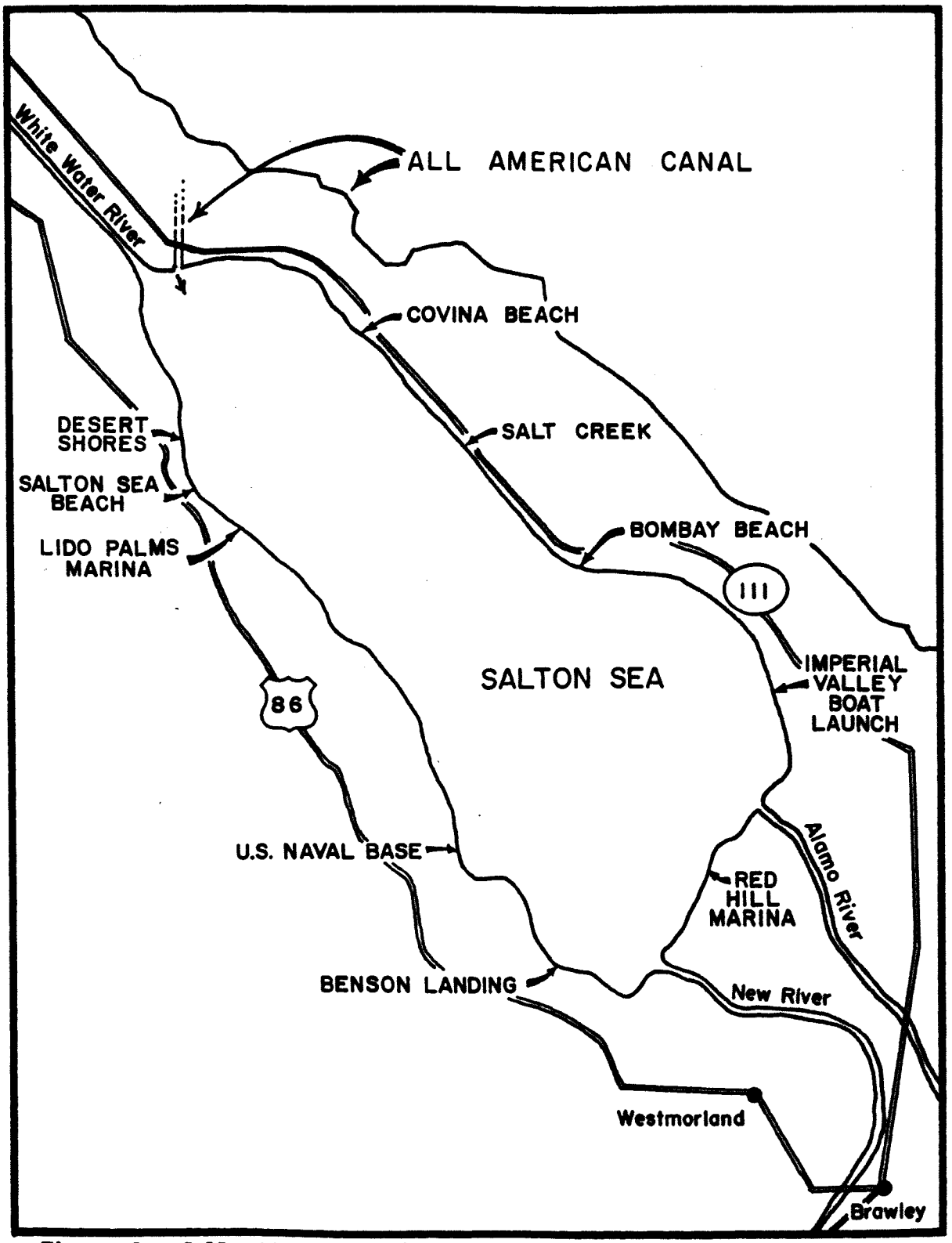

Figure 2. Collecting sites (denoted by shoreline arrow points). Adapted from Anonymous, 1966.

TAXONOMY OF THE FAMILY THRAUSTOCHYTRIACEAE

DIVISION: Eumycota. Thallus lacking chlorophyll; plasmodial, or unicellular and with or without rhizoids, or consisting of rudimentary hyphae, or of well-developed ones which may be septate. Asexual reproduction accomplished by endogenous or exogenous formation of motile spores, nonmotile spores of various types, or by budding and fragmentation, or by formation of resting or resistant spores. Sexual reproduction accomplished by fusion of motile or nonmotile gametes or gametic nuclei, the products either exogenous or endogenous, and borne in or on a unicellular or multicellular reproductive organ.

CLASS: Phycomycetes. Hyphae, if present, nonseptate except to delimit reproductive cells, but if absent, thallus generally one-celled and holocarpic, or eucarpic with an assimilative rhizoidal system and reproductive cells; spores motile or nonmotile.

ORDER: Saprolegniales. Thallus holo- or eucarpic; when eucarpic, consisting either of a rhizoidal system or a tubular, extensive hyphal system in addition to reproductive cells; septa, when formed, delimiting reproductive units. Sporangia produced, sometimes proliferating internally. Planonts biflagellate; mono- or diplanetic; when diplanetic, primary planonts pyriform with anterior flagella, secondary ones reniform with laterally inserted flagella; mono-, di- or multicystic. Sexual reproduction, where known, by gametangial fusion.

FAMILY: Thraustochytriaceae. Thallus epi-endobiotic, eucarpic, monocentric, consisting of an epibiotic sporangium or resting

spore and an endobiotic rhizoidal system with or without apophysis. Planonts laterally biflagellate; escaping as motile or nonmotile cells. Resting spores, where known, spherical, smooth, and containing a centric or eccentric oil globule (Johnson and Sparrow, 1961).

KEY TO THE FAMILY THRAUSTOCHYTRIACEAE

A. Sporangia usually globose; epibiotic, with or without internal proliferation (renewal); thin or thick-walled; smooth. Rhizoidal system endobiotic; simple or branched; apophysis present or absent. Planonts laterally biflagellate; motile or nonmotile when liberated through irregular fissure in partially deliquescent sporangial wall. Resting spores, where known, epibiotic, spherical, smooth, and containing a single centric oil globule. . . .Thraustochytrium

B. Sporangia spherical or ovoid; epibiotic; not proliferating; thick-walled; smooth; inoperculate. Rhizoidal system endobiotic; branched; arising from a vesicular apophysis. Planonts laterally biflagellate; motile when liberated through apical, circular pore in sporangial wall. Resting spore not observed. . .Japonochytrium

KEY TO THE SPECIES OF THRAUSTOCHYTRIUM

Modified from Johnson and Sparrow (1961); and Sparrow, personal communication:

1. Sporangium internally proliferous, obpyriform or globose, wall thin.

    2. Sporangium narrow-based with one basal rudiment for proliferation.

        3. Zoospores non-flagellated at discharge; sporangium obpyriform; wall bursting and dissolving distally. . . . . T. proliferum Sparr.

        3. Secondary zoospores flagellated at discharge, often motile in sporangium; wall of latter persistent.

            4. Sporangium globose to subglobose, cytoplasm pale yellow to orange; zoospores escaping through a single puncture in wall. . . . . T. aureum Goldst.

            4. Sporangium obpyriform; cytoplasm colorless; zoospores escaping by several fissures in wall.

                5. Zoospores escaping by one fissure; sporangium 15-35 x 7-28μ; zoospores 3.5-6 x 2-4μ . . . T. motivum Goldst.

                5. Zoospores exploding through one or several tears in the quickly disappearing wall; sporangium 14-17 x 17-19μ; zoospores 4.5-5.8 x 2.5-3μ . . . . T. kinnei Gaertner

    2. Sporangium broad-based with 2 or more basal rudiments for proliferation; globose or subglobose; zoospores motile within sporangium, escaping by tearing away of distal wall. . . . . T. multirudimentale Goldst.

1. Sporangium not proliferating, wall thick (capsular) or thin; globose or subglobose.

    2. Sporangium thin-walled.

        3. Sporangia 10-15μ in diameter, aggregated in botryoid colonies; rhizoids well-developed, much branched, sometimes intertwined outside substratum; zoospores swimming away at dissolution or disintegration of wall. . T. aggregatum Ulken

3. Sporangia 5-20μ in diameter; not conspicuously aggregated; rhizoids poorly developed, little if at all branched; actively moving zoospores swimming forth at bursting away of upper half of sporangium wall. . T. globosum Kob. and Ookubo.

2. Sporangium thick-walled (capsular).

3. Zoospore rudiments non-flagellated when released from sporangium by dissolution of wall, remaining clumped until (1 hour or more) flagella develop; wall 3-5μ thick; rhizoids unbranched; strongly salt tolerant. . T. pachydermum Scholz.

3. Zoospores oozing from distal part of sporangium as a mass of immobile flagellated bodies which as the mass expands assume individual motility; wall 2-6μ thick.

4. Protoplasm bright orange-red under X100; rhizoids coarse, well-developed, much branched. . . . . T. roseum Goldst.

4. Protoplasm somewhat cream-colored; rhizoids delicate, unbranched or simply branched. . . . . T. visurgense Ulken.

ORIGINAL DESCRIPTION OF Thraustochytrium pachydermum Scholz

Description. The sporangium is globular, less frequently oblong-oval or egg shaped. Its diameter ranges from 15-30μ. The wall is smooth, 3-5μ thick, and decreased somewhat in its thickness with lower salt concentrations. The rhizoid is delicate and, as far as could be observed, unbranched, without apophysis. The zoospores are unflagellated during emergence from the sporangium, and after a few hours, become capable of movement as a egg-shaped or drop-shaped body with two laterally attached flagella of unequal length; the longer flagellum is directed forward during movement. The larger diameter amounted to 5-7μ; the longer flagellum is 15μ long or more. Permanent states were not observed. During swarming, the fungus behaves as follows: the sporangial wall swells more and more, thereby becomes broader, loses its round form and is increasingly harder to recognize. At the same time the zoospores in the sporangium appear ever more distinctly. They remain lying, first without flagella, balled together in clumps, in or next to the sporangial wall which is only still visible in parts, and often ruptured as well. After 2-3 hours, the zoospores begin a vibrating motion and detach themselves from the clump 2-3 minutes later as biflagellated swarmers. Their movement is more uniform than that of the Chytridian swarmers. It is found that 2 or 3 swarmers, still, during the swimming away, adhere to one another. Nothing could be observed, which could have allowed disclosure of a cyst growing or moulting of the spores before the biflagellar state, as with some dimorphous Saprolegniaceae (Scholz, 1958).

DESCRIPTION OF Thraustochytrium pachydermum Scholz
ISOLATED FROM THE SALTON SEA

Thraustochytrium pachydermum was isolated from shoreline waters at Salt Creek, Salton Sea, California, October 19, 1969. Sterile pine pollen and onion tissue were used as baits. The sporangia are spherical, smooth and without etching. Sporangia are 15-20μ in diameter. The wall is 3μ thick. Rhizoids are endobiotic, slightly branched, and up to 30μ in diameter from the center of the thallus. They are often not visible due to their small diameter. The sporangial wall appears to deliquesce at zoospore maturity. During zoospore cleavage, the sporangium increases in diameter by ca. 5μ. Spore masses remain intact until spore dispersal. Zoospores are 3 x 5μ, bean shaped, and laterally biflagellate. Immediately prior to dispersal, flagella mature and become active. The longer flagella are 12μ or more long, and are directed forward in movement. The trailing flagellum sometimes lies close to the zoospore and is scarcely visible, giving the appearance of a singly flagellate zoospore. Zoospore maturation (from visible cleaving until complete dispersal) is approximately 50 minutes to 1 hour. Approximately 3 minutes elapse from commencement to completion of zoospore dispersal. Neither rhizoids or thallus are evident after zoospore dispersal.

RESULTS

Table 1. Determination of Salton Sea salinity

Collecting site	water temp.	water density	salinity corr. to 15°C
Red Hill Marina	21°C	1.0295	>40.0 o/oo
Imperial Valley Niland Boat Launch	18°C	1.0280	38.5 o/oo
Bombay Beach	18°C	1.0285	39.1 o/oo
Salt Creek	20°C	1.0290	>40.0 o/oo
Corvina Beach	20°C	1.0295	>40.0 o/oo
Desert Shores	16°C	1.0295	39.6 o/oo
Salton Sea Beach	18°C	1.0300	>40.0 o/oo
Lido Palms Marina	18°C	1.0300	>40.0 o/oo
U. S. Naval Base	18°C	1.0295	>40.0 o/oo
Benson Landing	18°C	1.0295	>40.0 o/oo

Data recorded November 26, 1969

Table 2. Utilization of carbon sources

Explanation of symbols. Fifteen or more thalli present in each of 5 X400 microscope fields recorded as (+). Absence of organisms recorded as (-).

	Thalli and zoospores after 4 days	Thalli and zoospores after 6 days	Thalli and zoospores after 8 days	Thalli and zoospores after 10 days
Salton Sea broth plus .1% glucose	+	+	+	+
Salton Sea broth plus .1% glycerol	+	+	+	+
Salton Sea broth plus .1% Na-acetate	+	+	+	+
Control: Salton Sea water only	-	-	-	-

Table 3. Growth as a function of salinity

Explanation of symbols. Fifteen or more thalli or zoospores, jointly, present in each of 5 X400 microscope fields recorded as (++). Five or fewer organisms present in 5 X400 microscope fields recorded as (+). Absence of organisms recorded as (-).

	Thalli and zoospores after 4 days	Thalli and zoospores after 6 days	Thalli and zoospores after 8 days	Thalli and zoospores after 10 days
Salton Sea broth plus 1.5 concn. water	++	+	+	+
Salton Sea broth plus undiluted water	++	++	+	+
Salton Sea broth plus 1/2 concn. water	++	++	-	-
Salton Sea broth plus 1/4 concn. water	+	+	-	-
Salton Sea broth plus 1/8 concn. water	+	++	-	-
Salton Sea broth plus 1/16 concn. water	++	++	-	-
Salton Sea broth prep. with distilled water	-	-	-	-

Table 4. Reproducibility of isolation by bait culture

	pine pollen bait
Red Hill Marina	+
Imperial Valley Niland Boat Launch	+
Bombay Beach	+
Salt Creek	+
Corvina Beach	+
Desert Shores	+
Salton Sea Beach	+
Lido Palms Marina	+
U. S. Naval Base	+
Benson Landing	+

Date of collection: November 25, 1969

Table 5. Utilization of naturally occurring substrates

Pine pollen; Pinus sp.	Multiple growth per grain. 15 or more thalli per grain common.
Date pollen; Phoenix dactylifera L.	Multiple growth per grain. Appears more susceptible than pine pollen.
Onion tissue	Growth present, but not extensive. Visual detection is difficult.
Human hair	No apparent growth.
Insect exuviae (meal worm)	Substantial growth on connective tissue remnants, but not on the exoskeleton.

Table 6. Thalli present in generation curve stock culture

Field no.	No. of thalli
1	3
2	1
3	4
4	5
5	4
6	5
7	4
8	1
9	1
10	6
11	4
12	5
13	6
14	5
15	5
16	2
17	7
18	4
19	6
20	3
21	6
22	3
23	7
24	5
25	4

average no. = 4.2 per X400 field

no. thalli per ml = (k)(n)(100) = 1,693,860 = 1.694×10^6

Table 7. Numerical increase of thalli during generation period

	Replicate				
	1	2	3	4	5
No. thalli per ml at start (log_{10})	4.2253	4.2253	4.2253	4.2253	4.2253
Avg. thalli per field; 96 hours	17	11	7	16	8
Avg. thalli per ml; 96 hrs. (log_{10})	6.8361	6.6470	6.4507	6.8098	6.5087
Avg. thalli per field; 120 hours	15	9	8	9	8
Avg. thalli per ml; 120 hrs. (log_{10})	6.7818	6.5599	6.5087	6.5599	6.5087
Avg. thalli per field; 148 hours	19	6	6	7	9
Avg. thalli per ml; 148 hrs. (log_{10})	6.8844	6.3838	6.3838	6.4507	6.5599
Avg. thalli per field; 172 hours	10	8	9	8	8
Avg. thalli per ml; 172 hrs. (log_{10})	6.5056	6.5087	6.5599	6.5087	6.5087
Avg. thalli per field; 196 hours	8	6	8	7	6
Avg. thalli per ml; 196 hrs. (log_{10})	6.5087	6.3838	6.5087	6.4507	6.3838

Table 8. Determination of generations: 0-96 hours

Replicate	$\log A_o$	$\log A_n$	$\frac{\log A_n - \log A_o}{\log 2}$
1	4.2253	6.6470	8.7 gen.
2	4.2253	6.5599	7.7 gen.
3	4.2253	6.3838	7.2 gen.
4	4.2253	6.5087	7.6 gen.
5	4.2253	6.3838	7.2 gen.

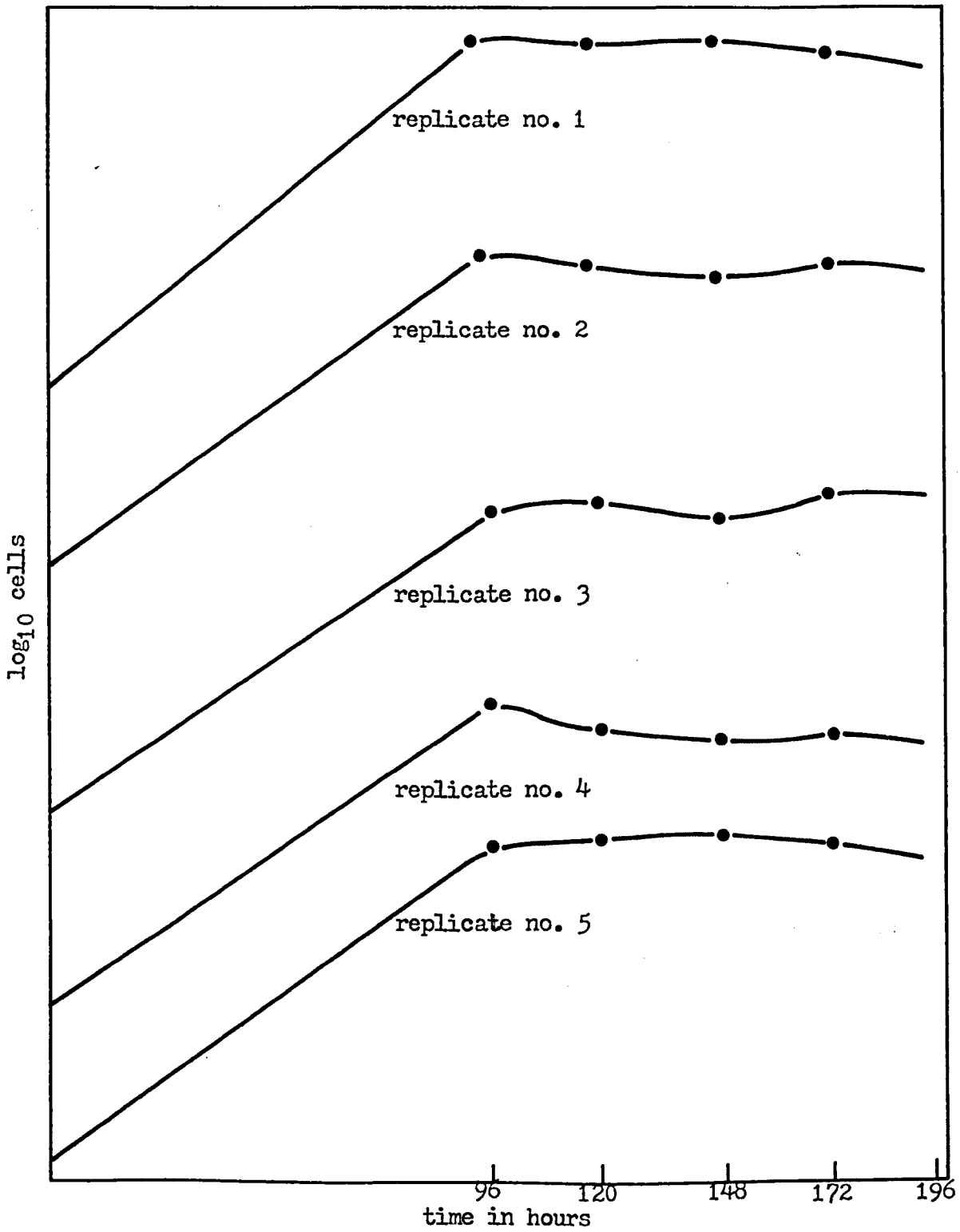

Figure 3. Logarithmic growth curve

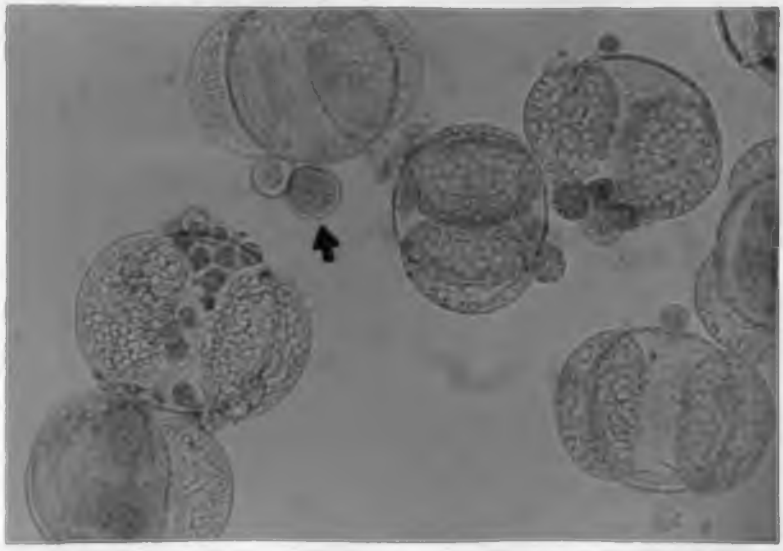

Figure 4. Pine pollen bait culture. Multiple infection. Largest thallus (arrow) equals 20μ diameter.

Figure 5. Sporangium cleaved; wall not visible. Sporangium is 20μ in diameter. Two smaller, independent, thalli subtend the sporangium.

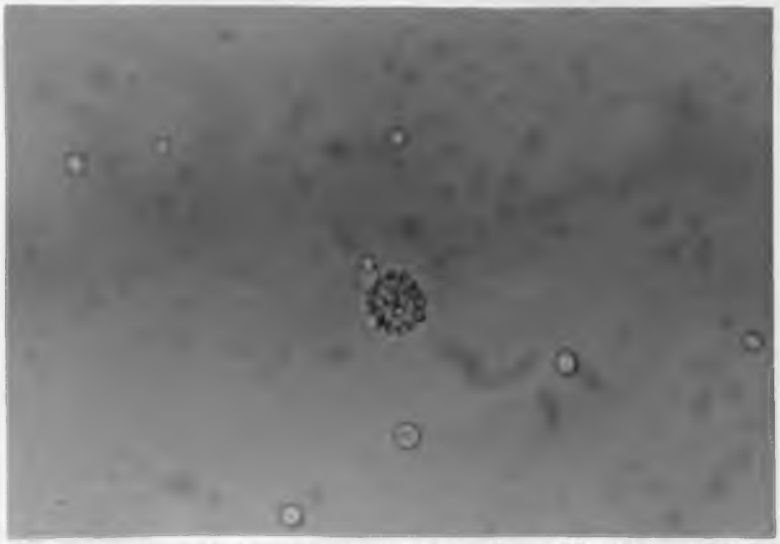

Figure 6. Mature sporangium immediately prior to zoospore dispersal. X400.

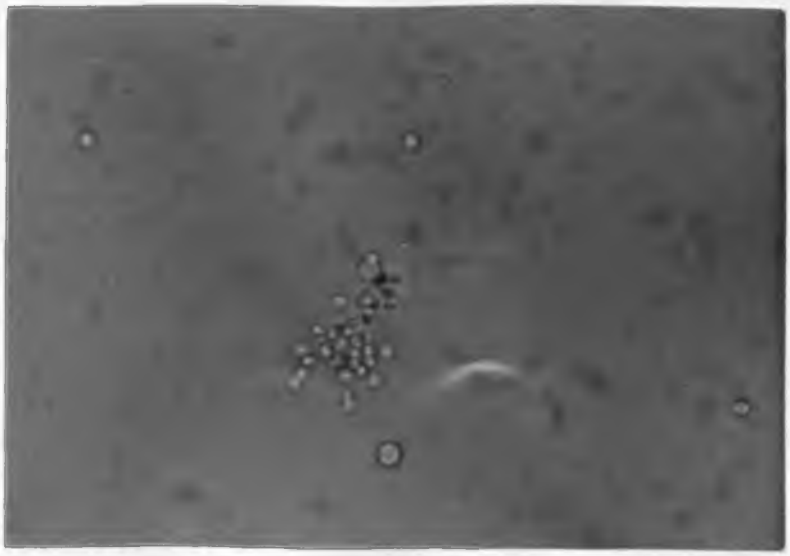

Figure 7. Same sporangium as Figure 6. Commencement of zoospore dispersal. Flagella not visible. Total time of dispersal, 3 to 4 minutes. X400.

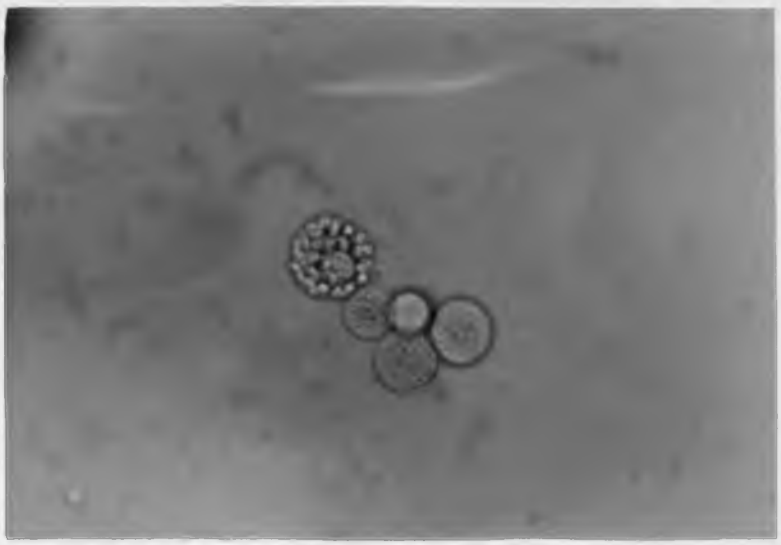

Figure 8. Cleaved sporangium subtended by immature thalli. X400.

DISCUSSION

T. pachydermum Scholz, isolated in this research, was initially assigned to the Class Phycomycetes on the basis of its lateral zoosporic biflagellate configuration (Alexopoulos, 1966). Of the biflagellate Orders representative to the marine environment, the following ones were eliminated for the morphological differences stated: Plasmodiophorales - endobiotic in its habitat; Peronosporales - produces planonts which mature exogenously in a vesicle; Lagenidiales - endobiotic in its habitat (Johnson and Sparrow, 1961).

Morphological differences between the original description by Scholz (1958) and the form in this study were not of great magnitude. The greatest variation noted is in the longer forward-directed flagellum which Scholz reports at 15μ or more while those forms herein bore flagella up to 12μ.

Scholz reported non-branching of the rhizoids. However, distinctly branching rhizoids were encountered in the Salton Sea isolates with regularity. Combined microscopic and photographic techniques were inadequate to record their fine structure. Sparrow does not believe differences in the matter of branching versus non-branching is critical as Scholz was not certain how much development his material had (Sparrow, personal communication).

The period of zoospore maturation could be followed, with certainty (Fig. 5, 6, 7), only about one hour preceding dispersal.

Immediately prior to dispersal, flagella mature, one followed by the other, and become active over a period of about one second.

Sex or sexual phases were not noted. Because Phycomycetes commonly exhibit a haploid life-cycle, the diploid state may occur as a zygote nucleus followed by reduction division (Raper, 1966).

Determination of the growth curve (Fig. 3), and its reproducibility over five replicates, reveals that the organism follows a regular exponential phase followed by deceleration, a stationary phase and the beginning of decline.

Growth on sodium acetate, glycerol and glucose as sole carbon sources was to be expected. As heterotrophs, fungi display broad carbon compound degradation (Hawker, 1966). Utilization of natural substrates revealed pollen to be most readily degraded. Because pollen contains both carbohydrate and protein sources, T. pachydermum would appear to be more restricted in substrate degradation than the Chytridiales, Blastocladiales or Monoblepharidales groups.

Several important facts were discovered from this study. First, glass vessels were employed in the generation determination to preclude the presence of a penetratable substrate whereby thalli could become sessile and unavailable for counting. The actual results showed that rhizoid affinity is not solely a matter of substrate penetration, but also includes the ability to attach onto hard surfaces, viz., glass.

Progress of the experiment resulted in both thalli present in solution (those counted in growth determination) and numerous thalli affixed to the glass resulting in a scum-like growth. Removal, and wet mount microscopic observation of the "scum" revealed hundreds of thalli.

That these were viable was not determined with certainty; however, vigorous agitation of the vessels did not result in added turbidity of the solution.

Secondly, growth curves are based on organisms present in solution (Fig. 8). Accordingly, it appears that an equilibrium does exist between sessile and free-living thalli. Because zoospores are motile, thalli probably begin, or are capable of, maturation in solution followed by growth and assumption of sessile residence.

Third, the reported growth curves are obviously not an absolute portrayal based on the aforementioned equilibrium behavior. Thus, generation times are shorter by some degree. Establishment of a more favorable environmental condition could possibly be realized by use of shake-culture techniques which may deprive the organism of its ability to become sessile.

Fourth, growth of T. pachydermum in varying salinities was important as the current thinking appears to follow a concept that it resides chiefly in or in proximity to salt encrustations (Scholz, 1958; Sparrow, 1968b). So far as is presently known, the organism has not been widely reported from the oceans or other localities of near oceanic salt content. This study represents the first known report of T. pachydermum from the Salton Sea.

Fifth, absence of growth on distilled water medium directly supports the work of Siegenthaler, Belsky and Goldstein (1967) where it was demonstrated that sodium was essential in the metabolism of Thraustochytrium roseum Goldstein.

CONCLUSIONS

The following conclusions may be drawn from this study: (a) the organism is widely distributed in the waters of the Salton Sea based on its numerous isolations; (b) T. pachydermum actively propagates in a habitat very near to the salinity of ocean waters; (c) morphology of the organism between high salt concentrations (Scholz, 1958) and the Salton Sea reveals stable character without dimorphic expression; (d) the organism does not require penetratable substrates for sessile residence; and (e) salts are necessary for growth.

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