Communications

Demonstration of Fumarase in Cell-Free Preparations from *Paramecium caudatum**

The presence of an enzyme catalyzing the hydration reaction

$$\text{Fumarate} \leftrightarrows \text{malate}$$

in cell-free preparations from *Paramecium caudatum*, variety 2, type IV (*1*) has been demonstrated by means of paper-partition chromatography. Other reported studies of enzymes of the Krebs cycle occurring in preparations from these organisms are limited to those of Humphrey and Humphrey (*2, 3*), who have reported the presence of succinic dehydrogenase on the basis of oxygen uptake studies with methylene blue as a hydrogen acceptor.

The organisms were grown in 4-lit serum bottles in 3 lit of culture mediums containing boiled wheat straw extract (50 ml), dried lettuce (50 mg), and dried skim milk (10 mg), which promoted a bacterial flora upon which the organisms lived. They were concentrated by inducing them to swim upward toward a light at a small orifice from which they could be bled off. The concentrated organisms were washed repeatedly with distilled water to remove the major bacterial contamination. The cell-free preparations were made in a Mickle tissue disintegrator (*4*) by vibrating from 2 to 3 min with several pieces of broken Pyrex. This produced 100-percent cell breakage with very little generation of heat. These preparations, made from suspensions of 10,000 to 25,000 organisms, contained from 50 to 200 μg nitrogen per milliliter. Nitrogen determinations were made by the method of Johnson (*5*).

Products of the reactions were determined chromatographically on Whatman No. 4 filter paper by the method of Lugg and Overell (*6*), using water-saturated n-butanol as the mobile phase, water as the stationary phase, and formic acid as a swamping acid to prevent ionization of the acids. All reaction mixtures were run in the presence of 0.02*M* phosphate buffer *p*H 7.4. They contained from 20 to 60 μg paramecia nitrogen per milliliter. Three runs were made with 0.05*M* fumaric acid as substrate. These flasks also contained $1.33 \times 10^{-6}M$ cytochrome c. Malic acid in each case was the only product detected. When 0.05*M* malic acid was used as substrate in the presence of 33 μg/ml DPN (diphosphopyridinenucleotide) fumaric acid was detected in each case, and in each case it was the only product detected. With malic acid as the substrate, even in the presence of 33 μg/ml DPN, 0.05*M* pyruvate, 15 μg/ml coA, $6.6 \times 10^{-6}M$ cytochrome c, and 250 μg/ml adenosine triphosphate, fumaric acid was the only product of the reaction to be detected chromatographically. In every case, the chromatogram of the reaction mixture at zero time showed only the presence of the added substrate.

The presence of fumarase in cell-free preparations of *P. caudatum* has been detected by the use of paper-partition chromatography. Further work to determine whether other enzymes of the Krebs cycle might be detected by application of chromatographic techniques is indicated.

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References and Notes

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13 September 1954.

A Fungus Flora of the Sea

During the past 100 years, occasional curious individuals have sought fungi in salt water. If we discount unpublished and therefore unknown failures, we see that they were generally successful in their searches, but we also see that those searches were not followed up with the enthusiasm that has characterized studies on the taxonomy and ecology of other special groups. In a resumé of the subject, Wolf and Wolf (*1*) say, "Among students of fungi and marine biology generally, a knowledge of marine fungi is largely nonexistent."

The reasons for lack of interest are not apparent. All marine organisms are important in the theoretical study of evolutionary relationships, and, according to Vishniac (*2*), "Marine micro-organisms are so little touched that it is safe to predict generally interesting biochemical results from almost any investigation of their nutrition." Furthermore, marine fungi promise to be of great economic importance (*3*). This is not commonly recognized, although they have been indicted as active agents in the destruction of plant and animal materials, both living and nonliving: eel-grass (*4*), diatoms (*5*), sea weeds (*6*), wood and fibers (*7*), and crab (*8*) and bivalve (*9*) larvae.

In connection with a marine borer survey, I have been making frequent collections of wood samples submerged in Limon Bay at the Atlantic end of the Panama Canal and in Panama Bay at the Pacific end. Using as great caution as possible to prevent chance contamination, I have isolated a number of fungus species directly from woody tissue. Immediate micro-

scopic examination always revealed vegetative hyphae among the wood fibers (Fig. 1). In three cases, fungi were isolated from marine organisms, although I do not know whether the fungi were parasitizing the organisms or living saprophytically. Algae yielded a *Tritirachium* and a *Pestalotiopsis*, and a tunicate bore fruit-bodies of the Ascomycete that Meyers (*10*) has provisionally called "Form No. 2."

Most of the genera thus isolated are imperfect fungi, and some, perhaps most, of the species have been found previously on land or in fresh water. However, they can and do inhabit salt water, they grow well on sea-water agar, and they are easily obtained throughout the year in this latitude. The genera in cultivation are *Aspergillus, Tritirachium, Pestalotiopsis, Fusarium, Scopulariopsis, Phoma, Gonatorhodum, Mucor,* and some forms whose affinities are still unknown. Meyers (*11*) has also found *Halophiobolus* in my collections. So far, no species of *Penicillium* has been isolated, despite the extraordinary ability of the genus to tolerate wide osmotic variations. Several of the species collected here are apparently undescribed. They may simply be unknown land forms or they may be strict salt-water inhabitants. Specific determinations are being made.

One result of past marine fungus surveys that has retarded investigation is this common discovery, particularly close to shore, of forms already known from terrestrial habitats. These have been unanimously rejected as not "true" marine species, because they may also be found in habitats other than salt water. An interpretation of *true* that puts such a special meaning on the word is not strictly followed in other biological fields. To reject a terrestrial *Aspergillus* as a marine form because it does not occur exclusively in the sea is like rejecting the typhoid bacillus as a pathogen because it can live in water. One might, with equal justification, not accept *Aspergillus* as a terrestrial genus because it can be found in the ocean. A somewhat similar situation obtained in soil mycology in the early part of this century when the idea of a fungus flora of the soil had a doubtful reception until living hyphae were clearly demonstrated (*12*).

Sparrow (*13*) has emphasized that clear distinctions do not exist between aquatic, amphibious, and terrestrial fungi. There is certainly an active fungus flora in the sea; and although some of the species are halophiles, many are able to thrive and reproduce in salt water or out of it.

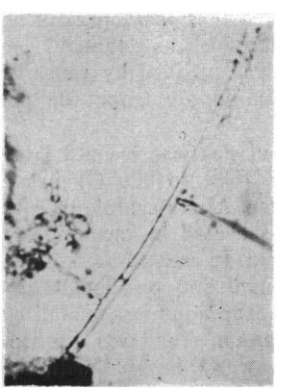

Fig. 1. Photomicrograph (×500) of vegetative fungus hyphae in fresh mount of wood, teased from a board submerged for 2 mo in Limon Bay, Panama, about 2 ft below the low-tide level.

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Submarine Photography in Puget Sound

There is no published information available on the operation of submarine bottom cameras from shipboard in the Puget Sound region. The present study (*1*) was undertaken to determine the feasibility of obtaining bottom photographs and to ascertain the most successful techniques to use. Subsequently, it is planned to utilize bottom photography as an aid in examining the bottom during biological, geologic, and physical studies.

Immediate use of the photographs obtained during this study is being made in an engineering study for the 230-kv cross-Sound electric power cable that is being contemplated by the Bonneville Power Administration. Depths of more than 700 ft at the 3½-mile crossing site just north of Seattle make direct examination of the bottom difficult. A second use has been found in assisting in the enumeration and identification of bottom-living organisms and in correlating the results of various bottom-sampling techniques near Anacortes, Wash. This is part of a study to determine biological conditions prior to establishment of industries that may cause pollution to the water.

An early Ewing type (*2*) shallow-water suspended assembly was strengthened for use in depths up to 1000 ft. A glass reflector (*3*) was substituted for the original reflector. The camera was a Robot rapid-sequence single-frame 35-mm camera with a Xenar *f* 2.8 lens. Plus x film developed in Microdal, No. 5 flashbulbs, and Kodabromide No. 4 enlarging paper developed in Dektol proved satisfactory. Results of numerous trials indicated that the most consistent results were obtained with the following camera and light settings: (i) aperture, *f* 8.0; (ii) exposure time, 1/100 sec; (iii) slant distance from camera to bottom,