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The *Butler University Botanical Studies* journal was published by the Botany Department of Butler University, Indianapolis, Indiana, from 1929 to 1964. The scientific journal featured original papers primarily on plant ecology, taxonomy, and microbiology. The papers contain valuable historical studies, especially floristic surveys that document Indiana's vegetation in past decades. Authors were Butler faculty, current and former master's degree students and undergraduates, and other Indiana botanists. The journal was started by Stanley Cain, noted conservation biologist, and edited through most of its years of production by Ray C. Friesner, Butler's first botanist and founder of the department in 1919. The journal was distributed to learned societies and libraries through exchange.

During the years of the journal's publication, the Butler University Botany Department had an active program of research and student training. 201 bachelor's degrees and 75 master's degrees in Botany were conferred during this period. Thirty-five of these graduates went on to earn doctorates at other institutions.

The Botany Department attracted many notable faculty members and students. Distinguished faculty, in addition to Cain and Friesner , included John E. Potzger, a forest ecologist and palynologist, Willard Nelson Clute, co-founder of the American Fern Society, Marion T. Hall, former director of the Morton Arboretum, C. Mervin Palmer, Rex Webster, and John Pelton. Some of the former undergraduate and master's students who made active contributions to the fields of botany and ecology include Dwight. W. Billings, Fay Kenoyer Daily, William A. Daily, Rexford Daudenmire, Francis Hueber, Frank McCormick, Scott McCoy, Robert Petty, Potzger, Helene Starcs, and Theodore Sperry. Cain, Daubenmire, Potzger, and Billings served as Presidents of the Ecological Society of America.

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PRESERVATION OF SOME ALGAL CULTURES BY LYOPHILIZATION

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Lyophilization has come to be widely used as a means of maintaining viable cultures of microörganisms in a desiccated state. Experimental application of this method of preservation in the algal group was therefore undertaken.

Raper and Alexander (1) believe that morphological and physiological characteristics of some of the fungi are not altered by such treatment. In their hands, strains of *Penicillium notatum* and *P. chrysogenum* preserved by lyophilization retained their original capacities to produce penicillin. Insofar as the algae cited in this paper are concerned, microscopic examination of the cells in viability check cultures indicated no morphological differences from the original cultures.

All but two of the cultures were supplied by Dr. Richard C. Starr of Indiana University. The Indiana collection number is affixed to each species.

CULTURE METHODS

The algae noted herein were cultivated on modified Chu No. 10 medium (2). The composition of the nutrient in grams per liter was as follows: $NaNO_3$, 0.124; $K_2HPO_4 \cdot 3H_2O$, 0.013; $MgSO_4 \cdot 7H_2O$, 0.025; Na_2CO_3, 0.020; $Na_2SiO_3 \cdot 9H_2O$, 0.058; $CaCl_2 \cdot 2H_2O$, 0.036; ferric citrate, 0.003; and citric acid, 0.003.

A temperature of 25°C was maintained and fluorescent tubes served as the light source.

LYOPHILIZING PROCEDURE

The algal suspensions were prepared by introducing 1 ml of sterile horse serum into the agar slant cultures. One tenth ml of each cell suspension was then dispensed into each of six sterile, cotton-plugged

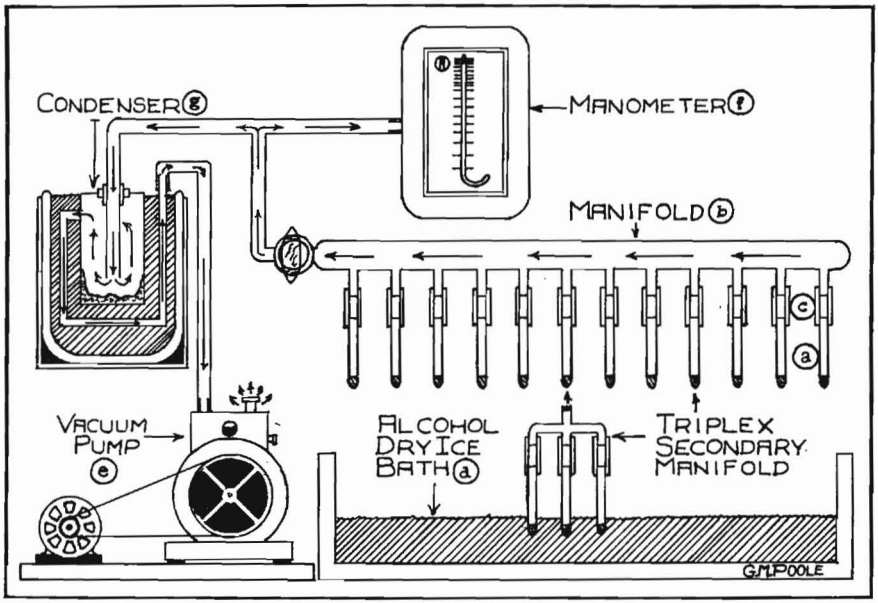

Fig. 1

(13 cm lengths of 7 mm Pyrex glass tubing, sealed at one end and lightly fire-polished at the lip) lyophil tubes by means of a sterile glass syringe fitted with a 9 cm 13 gauge syringe needle. The excess cotton on the plugs was burned off and the remainder pushed down into the tubes to a depth of about 1 cm as a precaution against possible contamination during processing.

The lyophil tubes (Fig. 1a) containing the algal suspensions were then attached to the manifold (Fig. 1b) of the lyophil apparatus by inserting them into the rubber sleeves (Fig. 1c). The manifold was then lowered into the bath (Fig. 1d) containing dry ice in ethyl cellosolve* until the bottoms of the tubes were submerged. The material in the tubes was completely frozen within a few seconds, and evacuation by means of vacuum pump (Fig. 1e) was begun immediately. After approximately two minutes (or until a minimum vacuum (Fig. 1f) of 150u of mercury is maintained) the temperature of the bath was raised from —55°C to —10°C. During the ensuing 1 1/2 hours, the temperature of the bath was allowed to rise slowly to about 0°C by which time chalky loose pellets were formed in the tubes. The moisture removed from the lyophil tubes is retained in

* Ethylene glycol monoethyl ether.

the condenser (Fig. 1g) where it collects as ice. The manifold was then raised from the bath and the vacuum was maintained for another hour at room temperature to insure thorough desiccation. The tubes were then sealed off *in vacuo* with a cross-fire gas-oxygen torch.

Within 24 hours or less after processing, the lyophil tubes were tested with a high-frequency, spark-coil tester to ascertain the existence of a vacuum.

Lyophilized cultures were checked for viability as follows. A lyophil tube was marked with a file scratch, swabbed with alcohol and broken open. The pellet was dissolved in sterile distilled water and the resulting suspension, appropriately diluted, was pipetted into agar plates and incubated for a period of 10 days, or until definite colonies developed.

No effort was made to modify any of the above processes, which are routinely employed for the preservation of other types of microörganisms in the Lilly Research Laboratories. Modifications in procedure and suspending agent could possibly be developed which would allow successful preservation of algal cultures which did not survive the process described here.

ALGAL CULTURES LYOPHILIZED

Chlorophyta

+8 Bracteococcus cinnabarinus (Kol. & F. Chodat) Starr #56
— Carteria crucifera Korsch #432
+7 Chlorella luteoviridis Chodat var. lutescens Chodat #248
— Chlamydomonas orbicularis Pringsheim #218
+ Chlamydomonas pseudococcum Lucksch #214
+10 Chlorella protothecoides Krüger #25
— Chlorella vulgaris Beijerinck #263
+4 Chlorella vulgaris var. viridis Chodat #30
— Chlorococcum macrostigmatum Starr #109
+ Coccomyxa elongata Jaag #267
— Cosmarium formulosum Hoff #303
— Haematococcus lacustris (Girod.) Rostaf. #16
+4 Muriella aurantiaca Vischer #36

+ Prototheca chlorelloides Krüger #178
+ Prototheca moriformis Krüger #288
+ Prototheca portoricensis Ciferri & Ashford #289
+4 Scenedesmus dimorphus Kütz. #417
+4 Scenedesmus dispar Bréb. #414
+4 Scenedesmus Naegeli Chodat #74
+7 Scenedesmus obliquus (Turp.) Krüger #78
— Selenastrum minutum (Naeg.) Collins #326
+ Stichococcus bacillaris Naeg. #419

13—BUTLER BOTANICAL STUDIES

Chrysophyta

+4 Polyedriella helvetica Pascher #49
+4 Vischeria punctata Vischer #153

Bacillariophyceae

— Navicula minima Grun. #391

Cyanophyta

+ Lyngbya sp. #487
+ Lyngbya sp. #488
+ Lyngbya versicolor (Wartm.) Gom. #29V Lilly
+ Nostoc sp. #387
+ Nostoc sp. #389
+ Nostoc ellipsosporum (Desmaz.) Rabenh. ex. B. & F. #27E Lilly
+ Phormidium luridum var. olivace Boresch #426

Legend:

+ = viable when tested within 24 hours following lyophilization.
+8 = viable at least 8 months after lyophilization.
— = non-viable immediately after lyophilization.

RESULTS

From a total of 32 cultures lyophilized, 24(75%) were viable when tested within 24 hours following desiccation. These continue to be checked periodically for viability. Duplicate lyophil tubes were cultivated recently, at from 4 to 10 months after lyophilization and they were still viable.

No spores or resting cells were seen in any of the cultures.

The percentage of cells of three species remaining viable after drying, determined by plate colony counts before and after drying was as follows:

Culture Lyophilized	Pct. Survivals
Scenedesmus obliquus	0.025% viable cells
Bracteococcus cinnabarinus	3.166% " "
Chlamydomonas pseudococcum ..	0.013% " "

CONCLUSIONS

The percentage viability of algal cultures withstanding lyophilization compares favorably with that of other groups of microörganisms, viz., bacteria, actinomycetes and molds when dried by a similar procedure.

The percentage of algal cells in 3 cultures remaining viable after desiccation, compares favorably with some bacteria (3).

This study demonstrates the practicability of lyophilization of the algae as a method of preserving certain algal cultures. If all algal cultures to be lyophilized are in excellent vegetative or sporiferous condition, a higher percentage viability rate probably can be achieved.

ACKNOWLEDGMENTS

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