JOURNAL OF PHYCOLOGY

OFFICE OF THE EDITOR

Department of Botany and Plant Pathology
Purdue University
West Lafayette, IN 47907
Carole A. Lembi, Editor
317-494-7887
FAX 317-494-0363
TELEX 4974301 INTAGPU

February 28, 1994

Dr. Joseph D. Andrade
Department of Bioengineering
2480 MEB
University of Utah
Salt Lake City, UT 84112

Dear Dr. Andrade,

Enclosed are two reviews of your manuscript (#93-196) on "Air drying a bioluminescent dinoflagellate (*Pyrocystis lunula*): feasibility study."

The reviewers seem to agree that the paper addresses a relevant question. However, there are a number of problems that need to be solved before the paper can be published. Both reviewers note the potential for toxicity upon drying down as the salinity and/or DMSO is concentrated during evaporation. Reviewer #1 indicated that it would have been more reasonable to start with lower concentrations of additives. Additional experiments would be helpful; at the very least, this potential problem needs to be mentioned. Reviewer #2 cited a number of technical problems and called for more in-depth explanation of methods. I also read the manuscript (my comments are in blue; red on the cover sheet) and found a number of concerns that must be satisfactorily addressed:

1. Please use *Journal of Phycology* format throughout; use a recent issue as a guide.

2. There is a range of extremes in how some aspects are handled. Part of this may come from an unfamiliarity with how algal research is communicated. At one extreme is the description of dinoflagellates on page 3. The vast majority of readers know what dinos are. This sort of description would be appropriate if you were writing something general for student use, but not for professionals. On the other hand, your description of some of the possible reasons for your results (e.g. on page 6) is much too detailed for the evidence at hand. Your results are fairly simple observations; they were not conducted at the molecular/cellular levels. Therefore, speculation about phase transitions, lipid reorganization, and the like are out of place and inappropriate without better reasons for making the linkage. Some general statements might be in order, but without adequate justification and relevance to the actual results, such detail must be deleted.

3. You may have felt obliged to add the detail on mechanisms to fill out the paper, because very little of the text is spent in describing your actual results. Virtually nothing is said about Figure 5, which contains most of the data for the paper. Your description of cell morphologies is

A PUBLICATION OF THE

PHYCOLOGICAL SOCIETY OF AMERICA

very elementary and pretty much confined to the figure legends. This is not appropriate; you need to describe the cells in a sequential order in the text so that the reader can easily make the necessary comparisons.

4. Again, there are extremes in how methods are presented. You provide two digrammatic figures showing the normal versus slow air drying processes. Neither of these figures is needed because your written descriptions in the Materials and Methods are adequate in portraying the steps of the procedures. On the other hand, you provide no information on other critical pieces of information, such as length of slow drying, number of times the experiments were repeated, how viability was actually measured (i.e. how many cells recovered to get a rating of viable), etc.

5. The descriptions of the cell morphologies are inadequate. What are the spine-like projections at the ends of the cells in Figure 4b? In that figure more than just the cell membrane appears to be broken. In fact, it is impossible to see a "broken" cell membrane. The cell contents appear to be disorganized and dispersed in contrast to Figure 1. Furthermore, what do you mean by cell membrane? Cell membrane can be a general term for all membranes in the cell. Most botanists would probably use plasma membrane or plasmalemma for the outermost membrane of the cell. Is that indeed what was broken? How can the reader tell this by looking at the figures?

6. Figures 1, 4, and 6 should be reduced, trimmed and arranged and mounted on a single plate according to *Journal* format. A plate of halftones should be no more than 17.2 cm in width (two-column) or 8.5 cm (one column).

7. A number of additional questions and inconsistencies are marked on the manuscript.

In summary, this paper needs a major rehaul before it can be published. There is no question that the subject is an important one. The storage and availability of viable cells for teaching and research would be a significant contribution. But, the premise on which you base viability is only weakly supported (observation of bioluminescence) if you do not describe how you established the quantitative criteria for viability. Was bioluminescence in only one cell sufficient to deem the culture viable? In 50% of cells? In 70% of cells? In addition, the paper needs to be greatly trimmed; speculation regarding mechanisms needs to be eliminated or greatly reduced so that it clarifies rather than muddies the water. You do not need filler. The manuscript can be very short (published in NOTE format) and still be a significant contribution. Your findings as presently configured do not merit a long document. Figures can be deleted. The reviewers' comments need to be addressed.

If you choose to continue here, please return the **enclosed blue-marked copy**, a revised original plus one copy, the original figures, and your responses to the reviewers' comments, point by point, at your earliest convenience. I will read the revision and determine at that time whether it is acceptable for publication.

If you would like to submit a color or black and white photo for consideration as a *Journal* cover, please see the enclosed form.

You still have much work to do, but the result should be a much stronger contribution to the literature.

Sincerely,

Carole A. Lembi

Carole A. Lembi
Editor

JOURNAL OF PHYCOLOGY

OFFICE OF THE EDITOR

Department of Botany and Plant Pathology
Purdue University
West Lafayette, IN 47907
Carole A. Lembi, Editor
317-494-7887
FAX 317-494-0363
TELEX 4974301 INTAGPU

INSTRUCTIONS FOR SUBMITTING A COLOR PRINT FOR THE *JOURNAL* COVER

If you are interested in submitting a photograph for consideration for the cover of the *Journal* in the issue in which your manuscript will appear, please send a 5 X 7 inch (12.7 X 17.8 cm) or square color (or black and white if more appropriate) print mounted on stiff white paper or board (no larger than 8.5 X 11 inches). Include a brief legend of one to three lines on a separate sheet.

The print should not duplicate any figure in your manuscript but should add to or complement your article.

If your print is not chosen for the cover of the *Journal*, please consider the following. The Board of Trustees of the Endowment Fund for the Society is interested in using prints of phycological topics for a calendar and other items. All proceeds from the sale of these items will go to the Endowment which, in turn, supports graduate student travel awards, grants, and other scholarly activities. Prints so chosen will be appropriately labelled with the authors' names and the legend.

Please return this form with your print.

_____ I do not wish to have my print considered for anything but a Journal cover.
Return the print to me: _____ yes _____ no

_____ I do wish to have my print considered for Endowment activities (if not chosen for a Journal cover). Please provide the following information:

Signature of corresponding author _____

Phone number _____ FAX number_____

A PUBLICATION OF THE
PHYCOLOGICAL SOCIETY OF AMERICA

Rev. #1

Air Drying a Bioluminescent Dinoflagellate (Min and Andrade)

This is a reasonable paper with a reasonable objective, though without very positive results. The commentary on mechanistic considerations is particularly good, but certain basic points seem to have escaped the authors. They should consider the following questions, perhaps in their discussion of future directions for any follow-up work.

1. Direct exposure of their cells to high concentrations of additive may induce unnecessary osmotic injury. For example, it would be less damaging to expose cells to 1 M DMSO by first treating them with 0.25 M DMSO and then transferring them into 1 M DMSO.

2. However, since the objective of their research is to dry the cells, the logic of using an agent like DMSO at a concentration like 1 M seems faulty. If the volume of water in the solution is reduced by evaporation, such that the final liquid volume is brought to 10% of the initial liquid volume, then the concentrations of all dissolved solutes will rise by 10-fold, including solutes such as DMSO. Thus, 1 M DMSO (about 7% by volume) would become 10 M (about 70% by volume) after drying, a presumably fatal concentration. Even 1 M DMSO exposed to cells for 3 days under ordinary conditions, let alone under conditions of stress such as drying, would be expected to be very toxic. Thus, 0.01 M DMSO might have been a better choice.

3. What is meant by "never allowed to become completely dry"?

4. Bioluminescence and morphology may be reasonable things for students to observe, but may not represent viability. The ability of the cells to divide would unequivocally represent viability.

5. "Bound water" is a misnomer: there is no such thing!

6. I do not believe Figure 6 shows cells dividing: the figure looks to me more like cells that have fused together.

7. The authors ought to consider the possibility of genetically modifying their organisms to allow them to elaborate intracellular solutes that have been shown to protect against dessication in organisms that are naturally resistant, or permeabilizing the organisms to make them take up such solutes.

Rev. #2

Review of the manuscript "Air drying a bioluminiscent dinoflagelate (*Pyrocystis lunula*): feasibility study"
(by Min and Andrade, 1994)

This paper deals with a new and simple procedure to preseve viable microalgae for short periods of time, a very little developed field in applied phycology. At this regard, the paper may be considered as a relevant contribution. It also contains a complete set of references from which a clear discussion of biological processes occurring during desication is compiled. There exists however many doubts respecting to the method used to dry the algae. The main problem that needs clarification is the following:

Two drying methods are described:

a)-The NORMAL drying, where cultures and solutions are let to dry for three days.

b)-The SLOW drying, where cultures are filtered through filter papers and then the solutionn is also filtered.

In case a) the evaporation at room temperature is concentrating both algae and salts, something that does not happens in b). On rehydration with new medium in artificial sea water, a very high salinity may be achieved in a). These salinities must be given in the paper. In addition , some controls for viability and bioluminiscence should be also stressed for undryed algae under such salinity conditions.

The specific questions and comments are marked on red on the manuscript and their corresponding descriptions are listed below.

1) Author's name has to be given.

2) What happened after three days? How concentrate was the algae suspension? How was the salinity?

3) As stated above, after rehydration, what salinity was achieved and how can this affect to viability and bioluminiscence of Pyrocystis lunula?

4) What type of filter paper (cellulose, fiber glass.....)?

5) How long were the algae exposed to the additive solutions. It is not clearly described whether the additive solutions were also filtered or not. Anyway, it seems to be difficult to pass through the filter if they are already blocked by the cells.

6) If wet filter papers were tightly covered, how was the degree of drying? An estimation of it should be made.

7) What does this means? Please quantify the amount of water and solute concentration.

8) Absence of viability is very likely to have occurred because the extended exposure to increasing osmolalities (up to an unknown level but important to know) during the normal drying system. From the first paragraph of Results and Discussion a misleading conclusion is obtained since only initial additive concentrations are considered and their build up produced by evaporation is not taken into account.

9) stresses destabilizing.

10) How can DMSO improve trehalose penetration. Please explain briefly.

11) Not neccesary. Repetitive.

12) Which methods? Please refer.

13) This is a very important fact.

14) Why "seemed" to be toxic?

15) After how long exposure?

16) In Fig. 5, the recoveries shown for all additive combinations tested are not quantified. Thus, this Figure is of poor usefulness since the relevance of the method is going to relay on significant recoveries. Many authors state 50% as the minimum recovery to consider the method suitable. It is essential to know percentage recovery after rehydration.

How many replicates did exist per treatment in Fig. 5?

#2

Air Drying a Bioluminescent Dinoflagellate (Pyrocystis lunula): Feasibility Study[1]

Dong J. Min and Joseph D. Andrade[2]

Department of Materials Science and Engineering and
Department of Bioengineering, 2480 MEB
University of Utah, Salt Lake City, UT 84112, USA

Running title: Air Drying of Dinoflagellate

Submitted to
Journal of Phycology

Dec., 1993

1. To whom correspondence should be addressed.

[1] Received 22 December 1993.
[2] Address for reprint requests.

ABSTRACT

The marine bioluminescent dinoflagellate was air-dried under or using normal and slow processes. We used the preservation additives trehalose, sucrose, polyethylene glycol (PEG), and dimethyl sulphoxide (DMSO) to attempt to minimize desiccation damage. The normal air drying process produced highly altered cells with no bioluminescence after rehydration. Slow air drying produced more positive results; cells in 0.1M DMSO, 1% PEG, and 5% PEG survived up to 3 days after drying as assayed by rehydration and bioluminescence. We believe it is possible to further develop an air drying process for these organisms.

Key words: air drying, bioluminescent, desiccation, dimethyl sulphoxide, polyethylene glycol, trehalose

INTRODUCTION

The preservation of cells, plants, animals, and foods has been studied for a long time. The two most common storage methods are drying and freezing. The common drying methods are air drying, vacuum drying, and freeze drying. ~~There is now growing~~ interest in air drying because it is cheaper and simpler than the other dry processes.

Certain plants, like the desert Selaginella lepidophylla (resurrection plant), are able to dry out completely during summer droughts and then come back to life upon rehydration without apparent damage ~~(1)~~. Other examples of desiccation tolerance include pollen, ferns, mosses, yeasts, tardigrades, and algae (2). Such organisms generally contain large amounts of trehalose, ~~a~~ an α-disaccharide of glucose ~~(2, 3)~~. Trehalose synthesis and accumulation apparently ~~avoids~~ prevents desiccation damage (1, 3, 12, 16, 19, 21, 22, 23). Other protectants against desiccation include sucrose, polyethylene glycol (PEG), and dimethyl sulphoxide (DMSO). Those chemicals are also well known as cryoprotectants (4, 5, 6, 15).

Sucrose is commonly found in seed embryos and is a common cell osmolyte in many unicellular algae, certain salt-tolerant plants, and many insects (2, 7). PEG is a good cryoprotectant for some proteins and was more inhibitory to yeast growth than was sucrose at a similar water activity (5, 6, 9). As aqueous solutions of PEG are hospitable to living cells, it is often utilized in tissue culture media and for organ preservation (27).

Use journal format: (Roser 1991)

Leopold 1990, (Crowe et al. 1992)

What is the significance of this?

DMSO is used as a penetrating additive because it penetrates membranes and enters both plant and animal cells (4, 14). Morris found that using an additive with a high permeability is not as damaging on an osmotic basis as one with lower permeability (14).

We studied the combination of trehalose and DMSO, hoping that ~~the~~ normally impermeant penetrating additives such as trehalose will be taken up more rapidly in the presence of DMSO (14).

We investigated the air drying of a marine dinoflagellate, a phytoplankton. Many marine dinoflagellates are bioluminescent, producing a blue light when stimulated at night (18). Dinoflagellates are single-cell organisms with both animal and plant-like characteristics, generally multiplying by cell division (10).

Well known by readers

The best additives for air drying depend on the properties of the organism. We wanted to study the air drying of single-cell dinoflagellates to hopefully minimize costs involved in the shipment of such organisms. We have developed dinoflagellate cultures which provide teachers and students with the experience of bioluminescence. To accomplish this we have to deliver the dinoflagellates within 3 days in order to keep the cells alive. Dry cells which could be easily reconstituted would greatly simplify the shipping problems. In this study we ~~chose~~ investigated the survival of the bioluminescent dinoflagellate Pyrocystis lunula after two types of air drying, normal and slow ...?

Rewrite something like this.

MATERIALS and METHODS

~~The dinoflagellate used in these experiments was~~ Pyrocystis lunula (Fig.1) was grown in ~~The basic~~ Guillard's F/2 culture media consisting of 0.5 ~~ml/l~~ vitamin working stock, 1 ml/l major elements, and 1 ml/l trace metals in artificial sea water (8). The ~~dinoflagellates~~ cultures were maintained at 20 °C with a ~~12/12h light/dark~~ 12:12 h L:D cycle.

In the normal air drying process ~~is shown in Figure 2.~~ P. lunula ~~Dinoflagellate~~ cultures were mixed with the additive solutions and then air dried in an open petri dish at room temperature for 3 days (Fig. 2).

The dried cells were rehydrated in culture medium and evaluated for growth and bioluminescence activity. The solutions used were 0.1M, 0.5M, and 1M trehalose (Sigma Chem. Co.); 0.1M, 0.5M, and 1M sucrose (Mallinckrodt Specialty Chem. Co.); 1%, 5%, and 10% PEG (average Mol. wt.=3400, Sigma Chem. Co.); and 0.1M, 0.5M, and 1M DMSO (Mallinckrodt Specialty Chem. Co.). We used culture media as an aqueous buffer for the solutions.

In the slow air drying process ~~is shown in Figure 3.~~ The cultures were first concentrated on filter paper (Fig. 3). The filtration step was repeated several times to obtain ~~high amounts of dinoflagellates.~~ large cell numbers. The additive solutions were poured slowly onto the dinoflagellates on the filter paper, thus bringing the dinoflagellates into contact with the solutions. After completely removing the excess solution, the wet filter paper with dinoflagellates was dried slowly in a petri dish covered tightly with plastic wrap for the desired time at room temperature. The same light cycle was maintained throughout

ml L^{-1}

irradiance? in µmol photons m^{-2} s^{-1} ADD

Is this a modification of Guillards? If not, then omit

how?

how do you know that the cells dried?

what times?

the "drying" process. The cells were never allowed to become completely dry. After storage, the partially dried dinoflagellates on the filter paper were rehydrated in culture media. The solutions were used ~~are~~ the same as for normal air drying. of what?

The resuspended cells were evaluated by their bioluminescence activity and by ~~study of~~ studying their morphology under the optical microscope. ~~Viable dinoflagellates could be recognized by their blue bioluminescence and by their morphology.~~ Redundant

How was bioluminescence activity measured? Visually? If so, how many cells (or %) had to be bioluminescent in order to be rated as survivors?

RESULTS and DISCUSSION

The normal air drying process (Fig.2) produced ~~very~~ altered cells with no bioluminescence ~~after exposure to all in any of the solutions and~~ after rehydration. ~~The morphology of the cells during drying is shown in Figure 4.~~ After drying for 2 days in 0.1M trehalose, the cells had shrunk (Fig.4(a)). ~~Also in the case of drying for~~ and after 2 days in 0.1M trehalose mixed with 0.1M DMSO, ~~we can see broken~~ cell membranes appeared (Fig.4(b)). ~~Similar results were obtained~~ With the higher concentrations of trehalose and trehalose mixed with DMSO, and the solutions of sucrose, PEG and DMSO, ~~we obtained similar results.~~

Water is a key component in maintaining the structure of membranes, nucleic acids, and proteins (11, 12). The three-dimensional structures of many biological molecules depend on the stabilization of the hydrogen bonding between water and macromolecules (1, 11). Membranes also depend on this complex bonding; the preservation of membrane interactions and structure is very important in research on desiccation. The preservation of microorganisms is closely related to maintenance of cell membrane structures during dehydration and rehydration. Desiccation of many microorganisms leads to large volume changes and metabolic unbalance due to changes in solute concentration, which occur during drying (2, 4). The cells often tend to fold and shrink (Fig.4(a)).

Two major stress destabilized membrane properties during desiccation are fusion and lipid phase transitions (3). When water is removed, the packing of the polar head groups in the membranes

tightens, which leads to an increase in the phase transition temperature (3, 5, 23). This phase transition induces the lipids to reorganize, which leads to the destruction of the membrane-layer geometry. Lipid reorganization leads to leakage during desiccation and rehydration. This leakage is due to an increased membrane permeability in the dry cell, caused by a shift in membrane phospholipids from a lamellar phase at high water activities to a hexagonal phase at lower water activities (3, 12, 16).

Non-reducing disaccharides, such as sucrose and trehalose, can maintain membrane structures because these sugar molecules replace structural surface-bound water and also prevent phase transitions in the lipid bilayer (3, 5). However, we did not have successful results when using these disaccharides, for several possible reasons.

First, trehalose is effective only when the disaccharide can be taken up by the cells (4, 13). If sugar molecules can not enter the cell, these molecules can not prevent the changes in membrane structure due to metabolic unbalance and water loss. We selected DMSO as a possible solution to the penetration problem. Unfortunately, there was not much difference with or without DMSO.

Plant cells, unlike animal cells, have rigid cell walls. the plant cell is therefore altered by large hydrostatic pressures, important for the movement of water and solutes into and out of the cell. For usual physiological conditions, a positive hydrostatic pressure exists inside a plant cell. The existence of the internal hydrostatic pressure leads to stresses in its cell wall. By adjusting the solute concentration in an external solution, the internal

hydrostatic pressure can be reduced. The volume of the membrane-bounded body changes in response to variations in the osmotic pressure of the external solution. This is a consequence of the properties of membranes, which generally allow water to move readily across them, at the same time restricting the passage of certain solutes, such as trehalose. If the osmotic pressure of the external solution were increased even further, a greater amount of water would flow out of the cell (26).

Relevance

11

Second, trehalose can be toxic to many plants (7, 11). ~~Configuration and steric factors are important in determining whether the action of a compound is toxic or protective to the cells (25).~~ We found that after keeping dinoflagellates in 1M trehalose for 2 days, most of the dinoflagellates died. However, this could be due to an osmotic pressure effect. ~~In the case of sucrose, if sucrose crystallizes, the hydroxyls will be unavailable, which causes mechanical disruption of the bilayer integrity.~~ Because of its tendency to crystallize, sucrose alone may not provide good membrane protection for desiccation (7, 11). Thus the choice of replacement additives is a critical factor in air drying.

this what? — why?

12

Direct application of the classical methods which ~~had~~ been ~~carried out~~ with animal cells and tissues was generally unsatisfactory for dinoflagellates. ~~From these results~~, the degree of protection ~~during desiccation~~ and the proper additives depend on many parameters, involving drying method and conditions, membrane composition, and geometry.

conducted have were all the above methods "classical"?

How do you know any of this worked? nothing to this point worked?

What are these? Define

How do we know that they are classical?

Anhydrobiotic organisms must dry slowly in order to survive (20). We therefore tried a slow air drying process (Fig.3), which

You have not shown this, i.e. that choice of additive has an effect, since none of the additives worked.

Indicate most successful treatments here

produced more positive results (Fig.5). Slower drying may provide time for a metabolic transformation essential to survival (2). Dinoflagellates in 0.1M DMSO, 1% PEG and 5% PEG survived 3 days after drying; these were the best results.

13

move description here

their what is their?

~~We may explain these results in two ways.~~ ~~One,~~ bound water is necessary for the maintenance of their structural integrity (2, 17). DMSO and PEG solutions hold more water than the other solutions. DMSO, which penetrates the membrane and enters the cell, may prevent dehydration of the cell by maintaining ~~the~~ internal water pressure (4). Morris found the high molecular weight additive polyvinylpyrollidone(PVP, average Mol. wt.=40,000) did not induce a significant loss of water from cells before freezing (14). From his results, we expect to produce the same behavior with PEG, which has a high molecular weight. PEG also has an amphiphilic nature, which suggests that it might interact with cell membranes. PEG-membrane interactions are probably involved in membrane fusion (27). The effect of PEG depends both on the numbers of molecules bound per unit area of cell surface and on the binding energies characteristic of this interaction (27).

Is there a reference for this?

3400- a far cry from 40,000 relative to what?

How can it be the same when normal air drying did not work at all?

The other reason is the same as in the case of normal air drying. DMSO produces (causes?) a pronounced increase in the phase transition temperature of phospholipid membranes, ~~indicating~~ (producing?) an increased stability (24). But a high concentration of DMSO, such as 1M, was not ~~good~~ effective; DMSO seemed to be toxic at such concentrations. (how do you know?) ~~In the case of~~ trehalose and sucrose, ~~the results~~ also were not ~~good.~~ effective as additives.

14

15

Figure 6 represents the cell morphologies after rehydration, following slow air drying. We see evidence of cell division, indicating the viability of these organisms after rehydration. The morphology of the cells in 0.1M DMSO was not greatly altered (Fig.6[a]), although there was some shrinkage in the 0.1M DMSO mixed with 0.1M trehalose, presumably due to the effect of trehalose (Fig.6[b]).

move to top of pg. 9

and evidence of cell division was observed.

cells exposed to

why?

CONCLUSIONS

We believe it is possible to air dry Pyrocystis lunula and related similar dinoflagellate. We need further research in order to find the optimum drying rate, the best additives, and the optimum concentration of the additive for each specific organism. Such techniques could have application for food storage and medical supply preservation.

no underline

is needed

ACKNOWLEDGMENTS

We would like to thank M. Lisonbee, C. H. Ho and Z. W. Gu for helpful guidance. Special thanks to Protein Solutions, Inc. for partial support of this work.

List references in alphabetical order

REFERENCE

1. Roser, B. 1991. Trehalose, a new approach to premium dried foods. Trends Food Sci. & Tech. July : 166-9.

Volume number?

2. Leopold, A. C. 1990. Coping with Desiccation. In Alscher, R. G. & Cumming, J. R. [Ed.] Stress Responses in Plants: Adaptation and Acclimation Mechanisms. Wiley-Liss, Inc., New York, pp. 37-56.

3. Crowe, J. H., Hoekstra, F. A. & Crowe, L. M. 1992. Anhydrobiosis. Annu. Rev. Physiol. 54:579-99.

etc.

4. Bhandal, I. S., Hauptmann, R. M. & Widholm, J. M. 1985. Trehalose as Cryoprotectant for the Freeze Preservation of Carrot and Tobacco Cells. Plant Physiol. 78:430-2.

5. Crowe, J. H., Carpenter, J. F., Crowe, L. M. & Anchordoguy, T. J. 1990. Are Freezing and Dehydration Similar Stress Vectors? A Comparison of Modes of Interaction of Stabilizing Solutes with Biomolecules. Cryobiology 27:219-31.

6. Carpenter, J. F. & Crowe, J. H. 1988. The Mechanism of Cryoprotection of Proteins by Solutes. Cryobiology. 25:244-55.

7. Caffrey, M., Fonseca, V. & Leopold, A. C. 1988. Lipid-Sugar Interactions. Plant Physiol. 86:754-8.

8. Guillard, R. R. L. 1975. Culture of phytoplankton for feeding marine invertebrates. In Smith, W. L. & Chanley M. H. [Ed.] Culture of Marine Invertebrate Animals. Plenum Publishing Corporation, New York, pp. 29-60.

9. Lenovich, L. M. 1987. Survival and Death of Microorganisms as Influenced by Water Activity. In Rockland, L. B. & Beuchat, L. R. [Ed.] Water Activity: Theory and Applications to Food. Marcel Dekker, Inc., New York & Basel, pp. 119-36.

10. Spector, D. L. 1984. Dinoflagellates. Academic Press, Inc., Orlando, 545 pp.

11. Koster, K. L. & Leopold, A. C. 1988. Sugars and Desiccation Tolerance in Seeds. Plant Physiol. 88:829-32.

12. Weisburd, s. 1988. Death-Defying Dehydration. Science News 133:107-33.

13. Coutinho, C., Bernardes, E., Felix, D. & Panek, A. D. 1988. Trehalose as cryoprotectant for preservation of yeast strains. J. Biotechnology 7:23-32.

14. Morris, G. J. 1980. Plant Cells. In Ashwood-Smith, M. J. & Farrant, J. [Ed.] Low Temperature Preservation in Medicine and Biology. Pitman Medical, New York, pp. 253-83.

15. James, E. R. & Farrant, J. 1976. Studies on Preservation of Schistosomula of Schistosoma mansoni and S. mattheei. Cryobiology 13:625-30.

16. Liljenberg, C. S. 1992. The effects of water defict stress on plant membrane lipids, Prog. Lipid Res. 31:335-43.

17. Crowe, J. H. 1971. Anhydrobiosis: An unsolved problem. Amer. Naturalist 105:563-574.

18. Hastings, J. W. 1983. Biological Diversity, Chemical Mechanisms, and the Evolutionary Origins of Bioluminescent Systems. J. Mol. Evol. 19:309-21.

19. Crowe, J. H., Crowe, L. M., Carpenter, J. F. & Wistrom, C. A. 1987. Stabilization of dry phospholipid bilayers and proteins by sugars. Biochem. J. 242:1-10.

20. Hand, S. C. 1991. Metabolic Dormancy in Aquatic Invertebrates. Advances in Comparative and Environmental Physiology 8:1-50.

21. Roser, B. 1991. Trehalose Drying: A Novel Replacement for Freeze-Drying. BioPharm. (Sept.):47-53.

22. Crowe, L. M. & Crowe, J. H. 1991. Stabilization of Dry Liposomes by Carbohydrates. Develop. Biol. Standard 74:285-94.

23. Crowe, J. H., Crowe, L. M. & Carpenter, J. F. 1993. Preserving Dry Biomaterials: The Water Replacement Hypothesis, Part 2. BioPharm (May):40-3.

24. Lyman, G. H., Preisler, H. D. & Papahadijopoulos, D. 1976. Membrane action of DMSO and other chemical inducers of Friend leukaemic cell differentiation. Nature 262:360-3.

25. Womersley, C. 1981. Biochemical and Physiological Aspects of Anhydrobiosis. Comp. Biochem. Physiol. 70B:669-78.

26. Nobel, P. S. 1974. Introduction to Biophysical Plant Physiology. W. H. Freeman and Company, San Francisco, 488 pp.

27. Harris, J. M. 1992. Poly(Ethylene Glycol) Chemistry. Plenum Press, New York, 350 pp.

List of Figures

Delete

} set up same as fig. 4

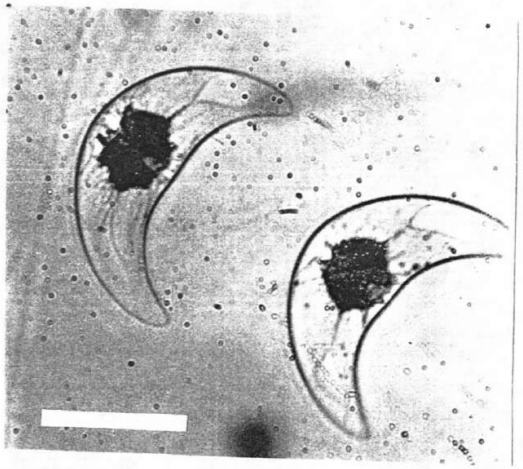


Fig. 1. Typical morphology of Dinoflagellate (Pyrocystis lunula). Bar: 0.1 mm.

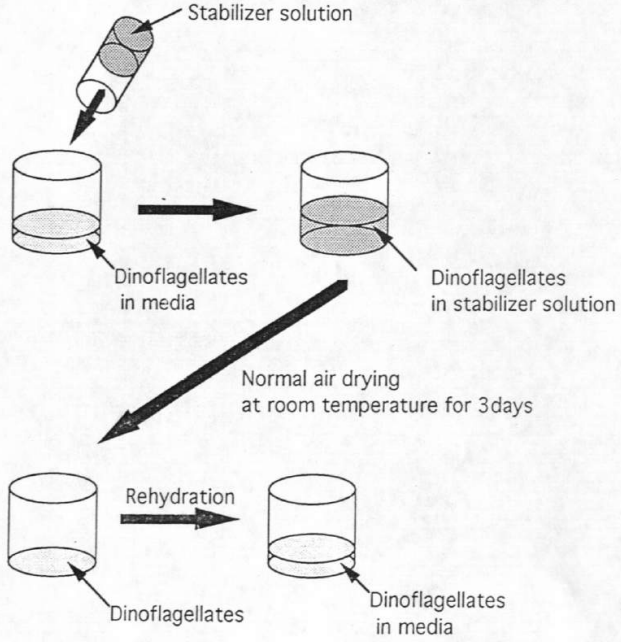


Fig. 2. The normal air drying process.

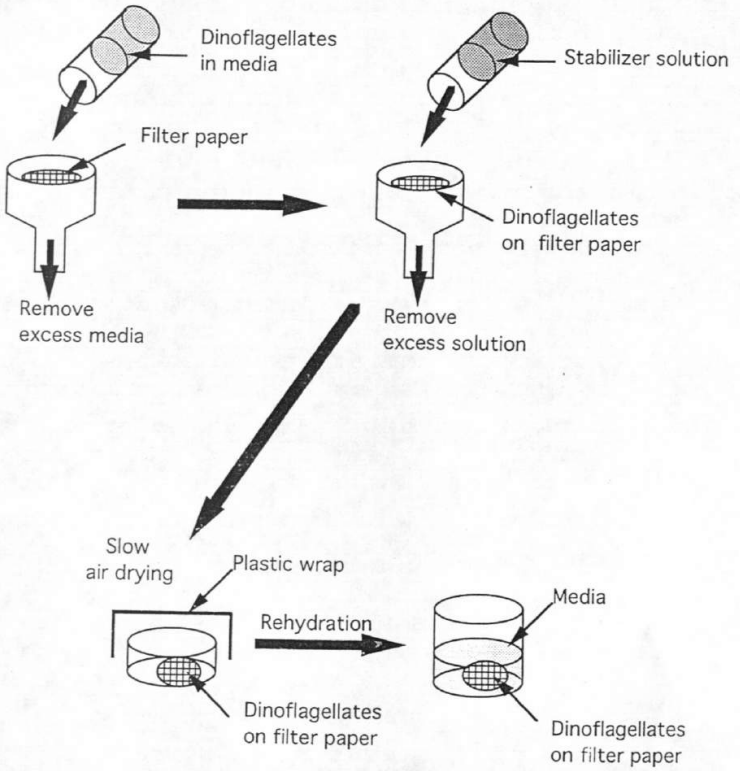
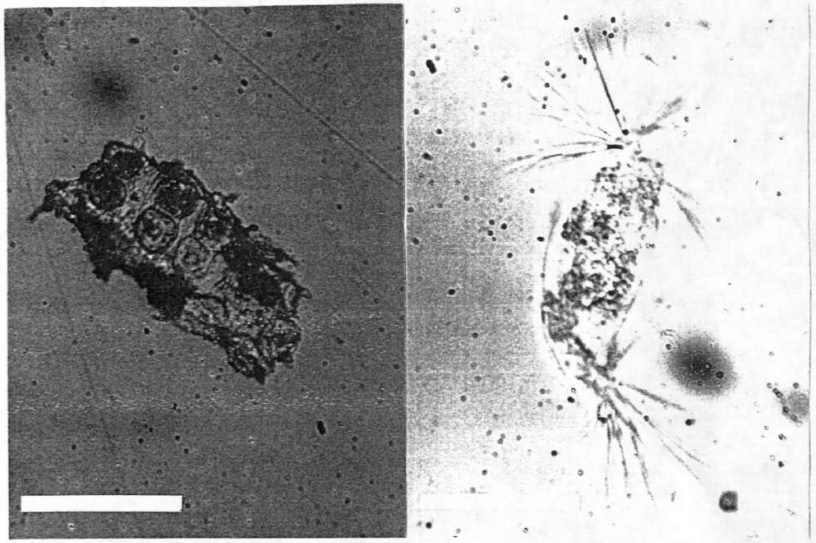


Fig. 3. The slow air drying process.



(a) (b)

Fig. 4. The morphologies during normal air drying after 2 days.
(a) In 0.1M trehalose: cell had shrunk with an altered, abnormal morphology.
(b) In 0.1M trehalose mixed with 0.1M DMSO: the cell membrane appears broken.
Bar: 0.1 mm.

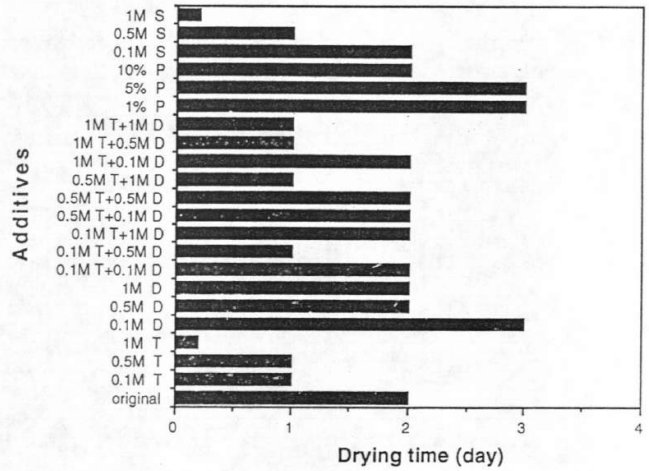
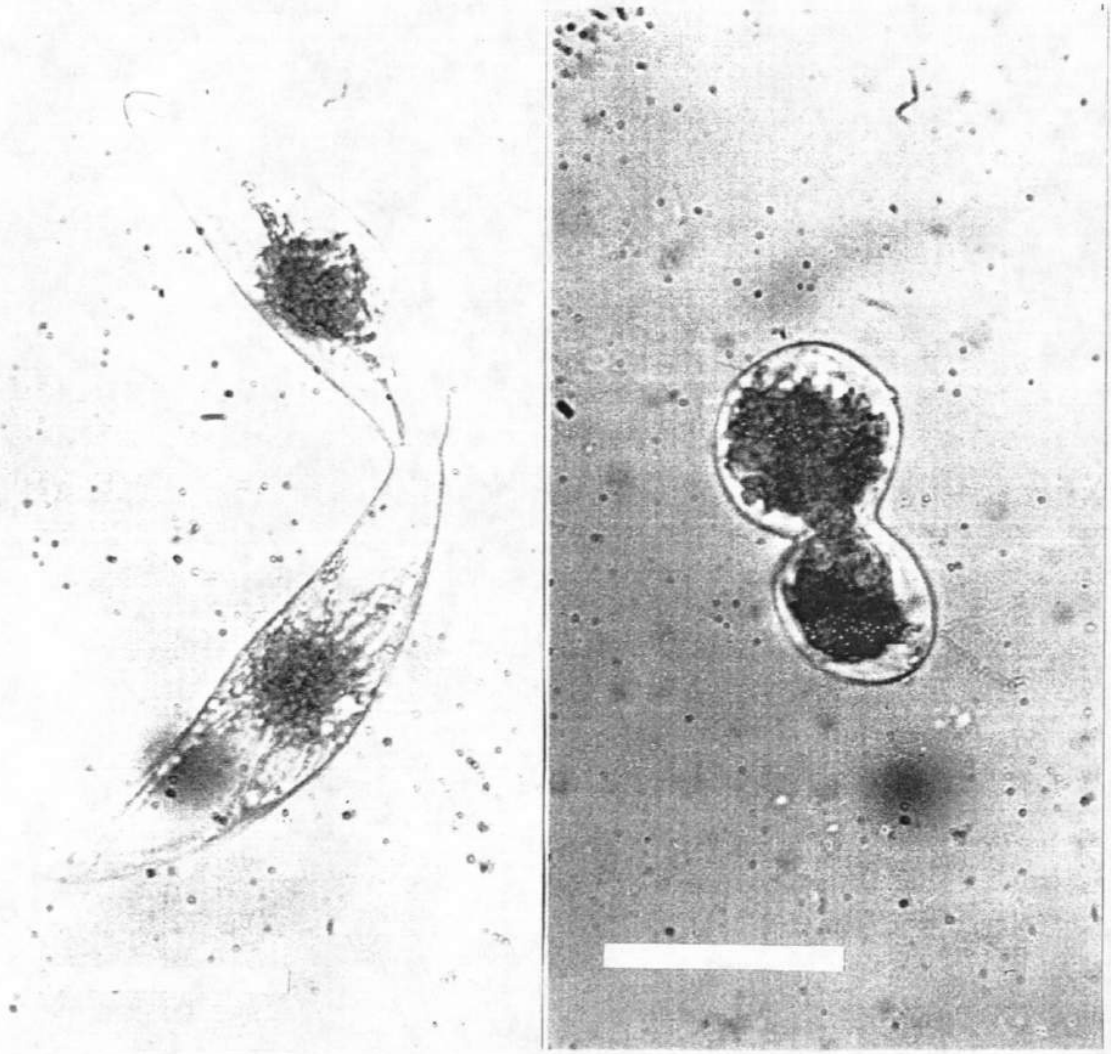


Fig. 5. The drying time from which Pyrocystis lunula recover after slow air drying.
S: sucrose; P: PEG; T: trehalose; D: DMSO



(a) (b)

Fig. 6. Pyrocystis lunula morphologies after rehydration after slow air drying for 2 days.
(a) 0.1M DMSO: apparent cell division.
(b) 0.1M trehalose mixed with 0.1M DMSO: apparent cell division with some shrinkage.
Bar: 0.1 mm