Maintenance of Cultures of Industrially Important Microorganisms[1]

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The great increase in number and size of industrial fermentations has accentuated the value of maintaining collections of microorganisms, especially of production strains, assay organisms and related species. Considerable work has been devoted to finding methods of maintaining cultures in a vigorous and stable condition. At present, the chief methods of preservation of industrially important microorganisms are by means of soil culture, agar slants, mineral oil overlay, and lyophilization. Each method has a place in any collection of microorganisms and all are in use in the collection of the Northern Utilization Research Branch, Agricultural Research Service, U. S. Department of Agriculture, at Peoria.

The NURB Culture Collection is organized in three parts. The first is devoted to bacteria with emphasis on the genera *Pseudomonas*, *Leuconostoc*, *Bacillus*, and *Streptomyces*. These cultures are designated by the prefix "B" with the culture numbers, and total over 2800 strains. The second part is concerned with yeasts and yeast-like organisms. These cultures, numbering over 2200, are designated by the "Y" preceding their specific number. The last portion of the collection consists of filamentous fungi with emphasis on species of *Penicillium*, *Aspergillus*, and the order *Mucorales*, though many additional fungi of economic importance are included. This mold collection numbers over 2400 isolates and can be recognized by the fact that no letter precedes the culture number.

No list or catalogue of our cultures has been published. The primary reason for this is that we do not wish to enter into competition with other collections.

In addition to the permanent cultures, the Collection has lyophil preparations of approximately 10,000 strains of microorganisms in pure culture which are not intended to be permanent accessions. However, because of the long viability of most lyophilized microorganisms, nearly all are still living and represent a reservoir of readily available material. They have not been incorporated into the collection because their usefulness has not been shown, or else they are known to duplicate cultures already in the collection.

The lyophil apparatus (figure 1) and the procedure used is essentially the same for all three groups of microorganisms (molds, yeasts and bacteria). A description of the apparatus and an explanation of the procedure were given by Wickerham and Flickinger (1946). It will not be amiss to emphasize two special advantages of the NURB process. The first of these is the relative inexpensiveness of the lyophil apparatus. The main item of expense is the vacuum pump. It, however, is of a type generally found in microbiological laboratories. The rest of the equipment consists of items which either are available in all laboratories or may be constructed fairly cheaply. The second advantageous feature is the speed of the operation. We can routinely lyophilize 30 cultures in triplicate in a day (3 runs with our setup). An experienced operator can increase production to 40 cultures in triplicate (4 runs) on any day that he is free of other duties.

The Bacterial Collection

The bacteria in the NURB Culture Collection were obtained, like the yeasts and molds, from other investigators and by isolation by ourselves and our colleagues. Not every strain received or isolated is kept, but those which have unique characteristics or are of known use in agriculture or industry are kept for preservation. For instance, a complete series of microorganisms of proven reliability in microbiological assay for vitamins and amino acids is in the collection. Strains selected for their fermentative capacities and described in publications by members of the USDA are retained. For example *Leuconostoc mesenteroides*, NRRL B-512F and related strains useful in dextran formation; NRRL B-72, *Acetobacter suboxydans* which makes sorbose from sorbitol, and NRRL B-6bs; *Pseudomonas sp.* which oxidizes glucose to 2-ketogluconate and α-ketoglutarate. Strains of known utility discovered and described by others are also sought.

If these were the only strains kept, the bacteria in the collection would number but a few hundred. The majority of strains are assembled because of their relationship to noteworthy strains. For instance over

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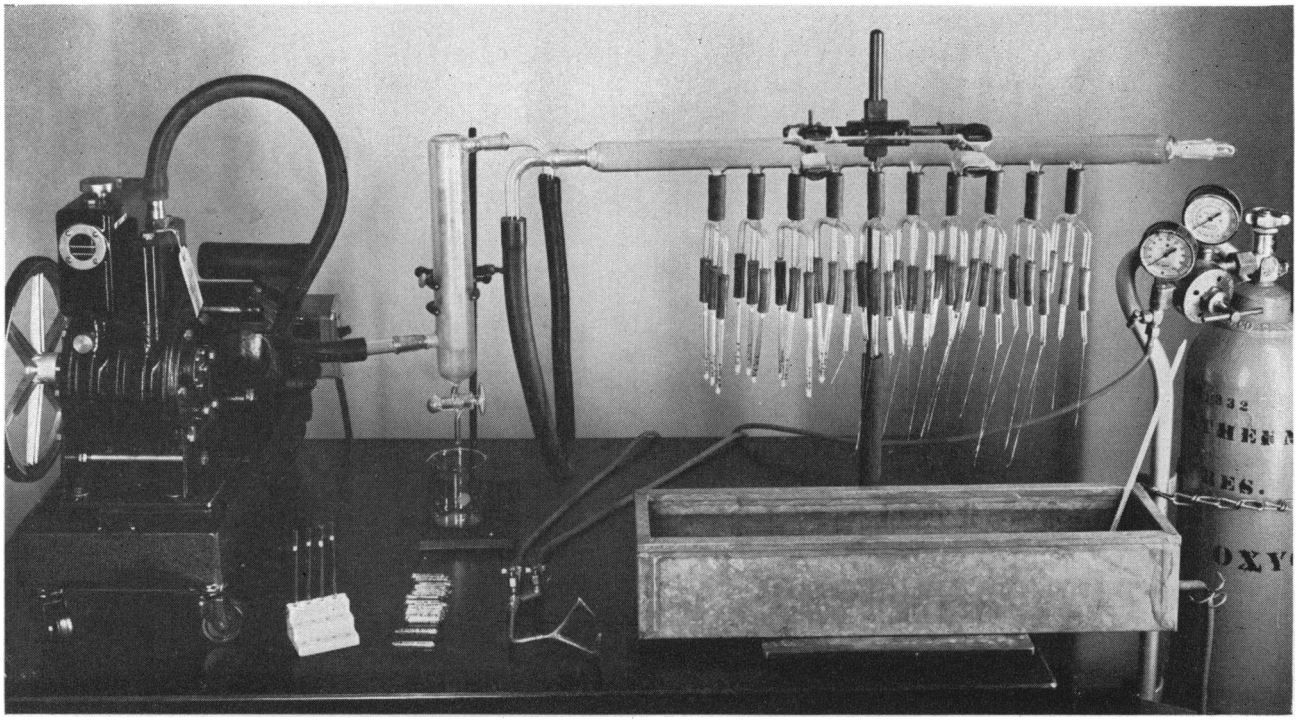

FIG. 1. A simple apparatus for lyophilization of bacteria, yeasts or molds

100 strains of leuconostocs which were acquired for comparison with NRRL B-512F are included. Another reason for keeping large numbers of organisms of unproven usefulness in agriculture and industry is to have a collection of type strains helpful in identification of isolates which prove to be interesting. Thus the NURB collection contains the N. R. Smith Collection of aerobic sporeformers. Of course, all of these unproven strains constitute a reservoir from which may be selected organisms having unique and useful properties.

Maintenance of 2800 strains even if not of diverse characteristics and requirements would be a tedious job. In the early days of the Collection all strains were carried on slants or in stabs and required transfer at least every three months, some strains even more frequently. Simultaneously with this method of maintenance, a program of lyophilization was carried on. This relieved us in large part of anxiety about losing strains, but we had not the courage for several years to rely exclusively on lyophilization. In the course of time, however, we became convinced that lyophilized bacteria were at least as safe as stocks kept in culture. Not more than one strain in a thousand failed to survive lyophilization. At length, we became aware of an apparently direct relationship between the numbers of cells subjected to lyophilization and the survivability of the preparation. Only in cases where the population was low did we experience failure. Furthermore, though we did not investigate the incidence of variation or mutation, we became convinced that this was not a problem with the organisms in our collection. On the contrary, we have several times recovered from lyophil a culture capable of yielding a characteristic product in quantities comparable to those recorded at the time of lyophilization, while the progeny of the same antecedent culture had declined radically in productivity. For this reason it has been our custom, whenever any microorganism performs exceptionally well in any of its activities, to reisolate the microorganism and to relyophilize it. By so doing we expect to preserve its activities unchanged.

For several years now we have relied exclusively on lyophilization for preservation of the bacteria including the actinomycetes. The only strains kept in culture are those in current use, and those which are frequently requested by other investigators. An exception to the general routine is the maintenance of the anaerobic sporeformers. Only a few such strains are included and they are kept in soil culture. Inasmuch as a single attempt to lyophilize them failed, it was not tried again. The streptomycetes and some of the aerobic sporeformers maintained in lyophil are also kept in soil culture.

Although we know of no *Streptomyces* which will not survive lyophilization, we do know that certain species in the genus have two prerequisites. They are firstly, that conidia must have been formed, and secondly, that the conidia must have matured. To insure sporulation we use several media, some of which are described in table 1. Having found one or more which gives good sporulation, we wait at least 14 days, sometimes longer, to allow the spores to mature before we lyophilize.

TABLE 1. *List of media used at the Northern Utilization Research Branch for the propagation and maintenance of the major groups of microorganisms*

I. Molds

A. Media especially suited for growing penicillia and aspergilli

Czapek's Solution Agar

Distilled H_2O	1000 ml
$NaNO_3$	3.0 g
K_2HPO_4	1.0 g
$MgSO_4 \cdot 7H_2O$	0.5 g
KCl	0.5 g
$FeSO_4 \cdot 7H_2O$ (1% soln.)	1.0 ml
Agar	15.0 g
Melt and add	
Sucrose	30.0 g

pH not adjusted.

Czapek's Solution Agar With High Sugar

The formula is the same as for Czapek's Solution Agar except that 20 or 40% sucrose is substituted for the usual concentration.

B. Media especially suited for the Mucorales

Potato Dextrose Agar (PDA)

Flask I

Distilled H_2O	100.0 ml
Dextrose	20.0 g
$CaCO_3$	0.2 g
$MgSO_4 \cdot 7H_2O$	0.2 g

Flask II

Distilled H_2O	400.0 ml
Agar	15.0 g

Flask III

Potatoes—peeled and sliced	200.0 g
Distilled H_2O	500.0 ml

Content of flask III brought momentarily to 121 C in an autoclave. Filter through cheese cloth. Bring up to original volume. Simultaneously the agar in flask II is melted and the solution in flask I is heated to boiling. Mix contents of three flasks.

pH not adjusted.

Malt Extract Agar

Distilled H_2O	1000 ml
Malt extract	20.0 g
Peptone	1.0 g
Dextrose	20.0 g
Agar	20.0 g

pH not adjusted.

C. Media especially suited for growing *Chaetomium* and some other Ascomycetes

Yeast Extract Agar

Yeast extract	4.0 g
Malt extract	10.0 g
Dextrose	4.0 g
Agar	15.0 g
Distilled H_2O	1000 ml

pH not adjusted.

II. Yeasts

One medium suitable for growing the majority of yeasts

MY Agar

Yeast extract	3.0 g
Malt extract	3.0 g
Peptone	5.0 g
Glucose	10.0 g
Agar	20.0 g
Distilled H_2O	1000 ml

pH not adjusted.

III. Bacteria

A. General purpose medium suitable for maintenance of the majority of bacteria

TGY Agar

Tryptone	5.0 g
Yeast extract	5.0 g
Glucose	1.0 g
K_2HPO_4	1.0 g
Agar	20.0 g
Tap H_2O	1000 ml

pH adjusted to 7.0.

B. A medium for the propagation and maintenance of lactic acid bacteria and anaerobic sporeformers

Deep Liver Medium

Liver extract	100 ml
Yeast extract	5.0 g
Tryptone	10.0 g
K_2HPO_4	2.0 g
Glucose	5.0 g
Distilled H_2O	900 ml

pH adjusted to 7.4.

Before dispensing to test tubes, add a small quantity of dried liver particles to each tube.

The liver extract is made by adding 2 liters of distilled water to each pound of finely ground beef liver and heating in flowing steam until all the redness is gone from the liver. This will require about three hours during which the concoction should be stirred frequently in order to break up clumps. When the supernatant becomes yellowish fluorescent it is filtered through several layers of cheese cloth. The clear supernatant is then bottled and sterilized.

The residue which remains after the above extraction is spread on shallow trays and dried at 50 C. This constitutes the dried liver particles mentioned above.

C. A medium designed for the maintenance of acetic acid bacteria but useful also for the propagation of fastidious types in other genera

Acetobacter Medium

Tryptone	5.0 g
Agar	20.0 g
Liver extract	100 ml
Distilled H_2O	900 ml
After melting agar, add	
Glucose	20.0 g
$CaCO_3$	10.0 g

pH not adjusted.

Dispense while stirring constantly to insure equable distribution of $CaCO_3$.

TABLE 1.—*Continued*

IV. Media upon which sporulation of *Streptomyces* is most likely to occur

Asparagine Dextrose Agar (ADA)

Tap H_2O	1000 ml
Asparagine	0.5 g
K_2HPO_4	0.5 g
Beef extract	2.0 g
Adjust pH to 6.8–7.0	
Add agar	17.0 g
Dextrose	10.0 g

Bennett's Agar

H_2O (distilled)	1000 ml
Yeast extract	1.0 g
Beef extract	1.0 g
NZ amine A	2.0 g
Adjust pH to 7.3 with NaOH	
Add Bacto-dextrose	10.0 g
Agar	20.0 g

(J. Bacteriol. *57:* 142, 1949)

Oatmeal Agar

Rolled oats	65 g
H_2O (distilled)	1000 ml
Cook to thin gruel in double boiler, filter through several layers of cheese cloth and bring up to volume.	
Add agar	20.0 g
pH not adjusted.	

Emerson's Agar

H_2O (distilled)	1000 ml
NaCl	2.5 g
Peptone	4.0 g
Yeast extract	1.0 g
Beef extract	4.0 g
Adjust pH to 7.0 with KOH.	
Add bacto-dextrose	10.0 g
Agar	30.0 g

(J. Bacteriol. *55:* 411, 1948)

It should be emphasized that there is no one culture medium which will induce sporulation of all strains and species of *Streptomyces*. In hopes of finding at least one that is satisfactory for this purpose with a given strain a variety of media are employed including asparagine dextrose agar, Bennett's agar, Emerson's agar, potato dextrose agar, Czapek's solution agar, oatmeal agar, yeast extract agar, starch agar, and corn steep agar. It is wise always to make sure cultures have an abundance of mature spores before storing them in a refrigerator.

Aside from the differences in stock-culture media, streptomycetes are handled more in the manner of molds than bacteria. In other words the culture is propagated by transfer of spores rather than of vegetative growth.

It is anticipated that a fourth category will be established to contain actinomycetes and other microorganisms such as algae and protozoa.

We have mentioned the fact that with few exceptions our lyophilized cultures have remained viable for as long as they have been under observation. Some of the tubes are 14 years of age. We no longer open tubes at specified intervals to check upon viability, but we record our observations on its condition each time such a tube is opened. Thus for most lyophil cultures a record of viability is available.

Having expressed supreme confidence in the lyophil method of maintaining and preserving stocks of bacteria, a word of caution should be expressed concerning sterility of the suspending agent. At NURB we use sterile bovine serum as the suspending medium for microorganisms to be lyophilized. The serum after separation is sterilized by Seitz filtration and then sealed in test tubes. The sterility of each batch is checked by opening selected tubes of serum and streaking on agar. It is not well to rely completely on the sterility of the serum thus controlled. Some very slow growing or psychrophilic organisms may be present and escape detection. It has been our practice to retest each tube of serum before it is used. This is done by spreading 0.5 ml of serum on duplicate plates of a nutrient agar and incubating them at room temperature for several days. Inasmuch as each tube holds sufficient serum for several runs, the same tube is entered repeatedly, and there is a danger that sterile serum may become contaminated. Since sterility testing uses so much serum, we prefer not to test it before each run but depend upon close observation. If any suspicion is aroused, the tube in question is not used until proven safe.

We maintain a minimum of four lyophil tubes of each strain, but those of special significance are made up to 10 or more. Four tubes are put into a vial which bears the strain number on its cap. These vials are arranged in trays which fit into a framework inside the refrigerator as shown in figure 2. The refrigerator temperature is set at 5 to 10 C.

No use has been made of oil-covered cultures for keeping bacteria because we have considered them unnecessary. For long-term preservation we are satisfied with lyophilization; for shorter term availability most of our bacteria survive for relatively long periods on artificial culture media. For instance, leuconostocs readily remain viable and unchanged in our deep liver broth for at least six months if kept under refrigeration following good growth. Pseudomonads, coliforms, aerobic sporeformers, and so forth, on agar slants will also survive for six months or more if kept refrigerated following good growth. If the plugs are burned off and

depressed and rubber stoppers inserted in the test tubes, viability may be greatly extended—to two years or longer with aerobic sporeformers.

Another reason for not using oiled cultures is that the first experience with this technique by one of the authors was unsatisfactory. Great variation among micrococci such as was never experienced with other methods of preservation was incited. Similar results have been reported in a collection of salmonellas (M. Caldwell, personal communication).

One of the problems facing anyone who tries to maintain a diverse collection of microorganisms is how to keep the number of media within reasonable bounds. We are fortunate in not having to face the difficult problems in nutrition attendant upon caring for a diversity of human and animal pathogens. Nevertheless we have organisms with different requirements and some of them are rather fastidious. For the vast majority of our strains a single medium suffices. For the lactics and anaerobes another is used while the acetic acid bacteria prefer yet another. In addition to these three media several others are suitable for special purposes or for special groups of bacteria represented by very few strains and in little demand.

When new strains are received or isolated, occasionally some difficulty in maintaining stability is encountered. The tendency to vary apparently is a response to new media and conditions to which the organism soon becomes accustomed. The degree of variation is sufficient with fresh isolates that cultures often are plated and colonies picked repeatedly in an attempt to purify a seemingly mixed culture. Some strains are so reluctant to adapt themselves to new media and conditions that they become troublesome. An example is NRRL B-512F which has been distributed far and wide for the production of clinical dextran. Most recipients of this culture apparently experienced no insurmountable difficulties in growing the strain and producing acceptable dextran. They probably either used the media and conditions recommended by us or, as inadvertently as we, were fortunate enough to select a suitable combination to begin with. A few, however, experienced a variety of troubles ranging from no growth to production of a dextran chemically and physically dissimilar to that produced at NURB and unable to meet specifications when prepared for clinical use. In most cases the matter was quickly amended when directions were sent to describe our methods and media.

As for the yeasts and molds, records are kept showing the accession number, date of deposit, donor and donor's identification. This information is filed chronologically, and alphabetically by donor and by genus and species.

The media upon which we rely most heavily for maintenance are given in table 1.

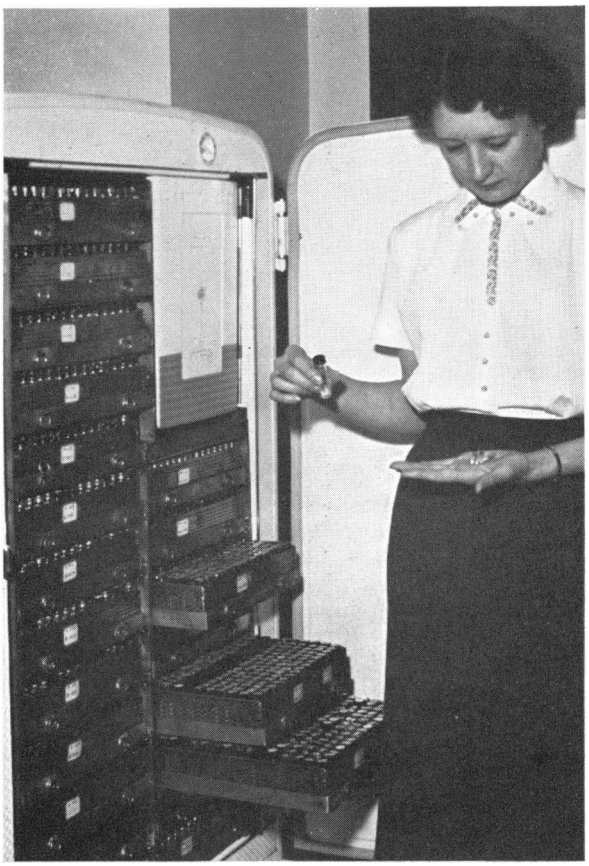

FIG. 2. Storage of lyophilized cultures

The Yeast Collection

Yeast cultures bearing specific names number over 2200. Most of these have been studied taxonomically. More than 3800 additional strains have been isolated from nature. Such isolates are lyophilized and their ability to sporulate is determined immediately after isolation. Their cultural characteristics are recorded and probable or definite generic placement assigned to them if possible. They are then available for developmental surveys or taxonomic study whenever the genus or general taxonomic area to which each belongs is to be intensively studied. About 90 per cent of the taxonomic work on yeasts at this laboratory is based on data obtained from isolates from nature.

Lyophilization of cultures was begun on a large scale in 1941, and all yeast strains have been preserved by this procedure. Each time a lyophil tube is opened, 2 or 3 more are prepared, until 5 are on hand, and the number is held approximately at this level.

Viability of yeasts in the lyophilized state is very good, and there is promise that investigators in the future may be assured that nearly all of the yeasts they lyophilize in their early years will remain alive without subsequent cultivation throughout their professional lives. Rare failures do occur with *Cryptococcus*, *Schizosaccharomyces* and *Saccharomycodes*. Occasionally

filamentous yeasts lose the ability to produce blastospores on solid media. These are grown in shake flasks where the mycelium develops as small dispersed elements that can be centrifuged and then successfully lyophilized.

Lyophilization of yeasts has never, to our knowledge, induced variation in any of the physiological characteristics upon which we base species identification. Quite to the contrary, for we believe it is the best means of preserving the characteristics of isolates. In fact, when one is studying a highly variable yeast and it becomes necessary to have certain phases as controls in each experiment, one may lyophilize the phases to be used as standards, then disperse the dried pellets in 1 or 2 ml of sterile mineral oil, contained in a screwtop vial, and streak a loop of the suspension each time a control is needed. We are indebted to Dr. F. M. Clark of the University of Illinois for this procedure.

Good results have been obtained in maintaining yeast cultures under oil. The medium used for both maintenance and general cultivation is MY agar which is described in table 1. The agar is solidified with the tubes in an upright position. The surface of the agar is inoculated and the cultures incubated 1 or 2 days at 25 C, then a layer of sterile mineral oil approximately 1 cm deep is placed over the growth. Then the cultures are stored at approximately 5 C, and transfers made at 2-year intervals. Some 1300 strains are maintained this way. A few which have failed to survive this period are transferred at 6-month intervals. A small number of osmophilic yeasts are kept on oiled MY agar containing 20 per cent glucose.

In our experience, preservation of yeasts in dry sterile soil has been very unsuccessful except for some mucoid and submucoid yeasts of the genera *Cryptococcus* and *Rhodotorula*.

The Mold Collection

When mold cultures are received or isolated, they are examined first of all for their purity and identified as completely as possible, or, if they are already identified, the identification is checked. It is also very useful, if the culture is not identified at the time it is secured, to have a record of what the appearance of the culture is under specified conditions. Thus, on a given medium at a certain age, a culture will have a characteristic spore color, reverse color and a soluble pigment present or absent. These characteristics may be determined readily with a minimum amount of labor. Later, should a question arise as to the appearance of the culture when first obtained, the information is available.

The filamentous fungi are routinely carried on agar slants, in lyophil, and in soil cultures. However, a few recalcitrant to lyophilization are kept under mineral oil. Wherever possible each permanent culture is carried by at least two of the above methods, usually in lyophil and on agar slants. The lyophil process has the advantages of allowing cultures to be kept for extremely long periods of time, the cultures are sealed and cannot become contaminated during storage and they require little space for storage. On the other hand, when a lyophil tube is opened, it may be necessary to re-lyophilize to replace the tube. Such recultivation and reprocessing requires considerable time. In some instances, as among the *Entomophthorales* and certain mucors, in spite of abundant spore production, poor viability or no growth at all follows lyophilization. There are probably other species with spores which fail to survive the process. For this reason, each time a lyophil culture is prepared, it should be checked for good growth within a period of one or two weeks to insure that the preparation is satisfactory. The process cannot be applied successfully to nonsporing cultures. It is generally believed that a large proportion of cells are killed by freeze-drying. In consequence there has been speculation that selective killing might cause variation in lyophilized cultures. In our experience, however, this has not happened.

The mold cultures are carried on a variety of stock media in the form of slants. Compositions of some of these media are given in table 1. The aspergilli and penicillia are carried on Czapek's solution agar, or malt agar; certain cultures are grown also on modifications of Czapek's solution agar. For example, *Aspergillus ruber* which requires a high-sugar content in the medium is carried on Czapek's solution agar with 20 to 40 per cent sucrose added. Members of the *Mucorales* have been carried for years on laboratory-prepared, potato-dextrose-agar with excellent success although there is reason to believe that malt agar or other nonsynthetic media might be just as good.

It is important to realize that some species have specific requirements for growth. Consequently one is forced to use specific media or techniques for many fungi. An interesting example of special techniques required for maintaining unique types of microorganisms is illustrated by the success one of us (CWH) has had in maintaining numerous members of the genus *Syncephalis* and *Piptocephalis*. These genera, which contain a large number of species and which are fairly common in soil, grow as obligate parasites on saprophytic *Mucorales*. None has been cultivated free from its host. Because we are interested in members of this order, we have secured a number of species and are collecting additional ones which we wish to maintain permanently. As seen in nature, they are found growing on a variety of host genera. The usual procedure in securing such a parasite is to isolate mycelium of the host and parasite, and then to purify it of extraneous bacteria and molds by repeated subculture. A more convenient method is to isolate the host from the plate by picking sporangiospores from a sporangium and,

after inoculation of three places on nutrient agar in plates, to inoculate the same spots on the agar with a few spores of the parasite picked from their sporangia, and usually the two then can be obtained in "mixed-pure" culture. However, in carrying stock cultures on agar, one is forced to check the culture carefully, after each transfer, to be sure that the host has sporulated and that the parasite's spores have germinated, attacked the host, sporulated in abundance, and matured their spores. For some time cultures were lost because the host, when freed from the competition of other microorganisms, grew so rapidly and luxuriantly that the parasite could not properly grow, with resultant failure to sporulate. Also the host might be of such a similar gross appearance that the absence of growth of the parasite might be missed and the culture lost. The maintenance of these cultures was greatly simplified when it was realized that one could use *Mucor ramannianus* as host for most species of the two genera. It is a red-colored *Mucor* with extremely short sporangiophores and velvety, slow-growing colonies. When a member of the genus *Syncephalis* or *Piptocephalis* is encountered in a soil sample or a culture is received with one of the parasites in it, we routinely inoculate plates with *M. ramannianus* and infect the cultures with spores of the parasite in the above manner. In most cases the parasitism of these genera is not so specialized that they will not attack this new host. When the "mixed-pure" culture is established, no difficulty is encountered in maintaining it. It has the distinct advantage that the host never overwhelms the parasite as *Rhizopus stolonifer* may do. It also enables one to see with the naked eye, because of the red color of the *Mucor*, whether the host has sporulated. Invariably, the parasitic species are much taller and one can tell at a glance whether or not the surface of the host has been overgrown and the parasite has fruited. Usually the parasite will grow over the host and far beyond on the surface of the agar, apparently translocating nutrients from the host. In these areas the parasite's mycelium and fruiting can be studied without the presence of the host mycelium and could be used for studies on the factors required for parasitic growth. Since we started to use this technique, not a single host-parasite combination has been lost.

Cultures are examined prior to storage for adequate growth and sporulation, for contamination by bacteria and molds, for reverse color, and for the general microscopic morphology as seen under a dissecting microscope. In some species it is particularly important to check for sectoring or production of sterile mycelium. Once a culture has degenerated to the point where no sporulation occurs, it is practically impossible to get it to appear again in the normal sporulating state. However, if this progressive degeneration is detected before it is complete, a vigorous, heavily sporulating culture can be reisolated which will appear as it did when first isolated. In some cases degeneration can be arrested by changing to a more favorable culture medium. Cultures however prepared are stored in refrigerators or cold rooms at 5 to 7 C. Each tube should bear the date of transfer, the culture number, the culture medium and the temperature of incubation. Before storage, each cotton plug should be poisoned to prevent mold growth and entrance of mites. Transfers are made at 6- to 8-month intervals.

It is also important that the chill room or refrigerator have a low relative humidity, otherwise undesirable molds will tend to grow on paper labels, and so forth, which makes contamination almost a certainty. A word should also be said about the care of the laboratory. Every precaution should be taken to prevent infestation of the work area with mites which occasionally may be introduced with cultures, soil samples or other material sent in from the outside. It is needless to say that all materials on which fungi have been allowed to grow should be carefully sterilized before washing to prevent heavy contamination of the general work area. When working with molds, especially those with dusty spores as *Rhizopus stolonifer*, the transfer needle should be sterilized and then moistened in sterile agar to prevent spores from being dispersed into the air. Also, with these cultures it is especially important that the fewest possible spores be used as inoculum. Whenever possible, one should transfer spore material only. In some cases a light touch of the needle on the sporulating area is sufficient. However, some fungi, including many of the wood decay fungi, require the removal of a block of agar and mycelium since no basidiospores or conidia are produced.

Summary

The basic principles which guide the maintenance of the NURB Culture Collection are:

The person in charge of a given category of microorganisms must be well enough acquainted with the component taxa that he will usually know the media and conditions best suited to permit typical growth of any strain. From more or less cursory examination of a culture, he must be able to decide whether it is typical, free from contamination, and in proper condition for distribution. In difficult cases he must have at his fingertips sources of information to resolve any questions.

The methods of preservation must be chosen from a knowledge of the advantages and disadvantages of the various methods and from a comprehension of the characteristics and requirements of the microorganisms to be preserved. Until a group has become well known, it is not advisable to depend on a single method of culture or preservation for maintenance.

The salient hazards of maintaining a collection must be known and guarded against. Among these are infestation with mites, contamination of cultures, especially with molds which grow at high humidities, misnumbering or mislabelling of cultures and so forth.

The NURB collection is devoted to organisms of industrial importance, especially in the field of fermentation. This specialization is emphasized in order to avoid overloading facilties with types not adapted to fermentation work.

The personnel of the NURB unit are required to have thorough training in the field encompassing the microorganisms in their charge. Interests in taxonomy are encouraged and every effort is made to see that opportunity exists to carry on a sustained taxonomic investigation of selected microorganisms.

About 1000 cultures per person is the proper assignment in maintaining the collection adequately. This, however, is dependent upon use of lyophilization for culture preservation.

REFERENCE

WICKERHAM, L. J., AND FLICKINGER, M. H. 1946 Viability of yeasts preserved two years by the lyophil process. The Brewers Digest, **21**, 55–59 and 65.

Methods for the Detection and Estimation of Numbers of *Salmonella* in Dried Eggs and Other Food Products[1]

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Dehydrated eggs are an important item of procurement for Armed Forces feeding. Because the presence of *Salmonella* in egg products constitutes a potential health hazard, it is obviously important to the Armed Forces that food infections and carrier states caused by *Salmonella* in eggs be prevented among troops. The purpose of this report is to describe improved test methods for estimating the extent of *Salmonella* contamination in foods in order to assist in the control and elimination of this food infection hazard. Many investigators (Gibbons and Moore, 1944; Schneider, 1946; Gibbons, 1947; Solowey *et al.*, 1947; Solowey and Rosenstadt, 1948) have reported the occurrence of these microorganisms in eggs, and McCullough and Eisele (1951*a*, 1951*b*, 1951*c*, 1951*d*) established the pathogenicity for humans of strains of *Salmonella* derived from spray-dried whole egg. Outbreaks of *Salmonella* infections which were traced to eggs have been described (Watt, 1945; Mitchell *et al.*, 1946; Medical Research Council, 1947). Edwards *et al.* (1948) state that "eggs and food products containing eggs may more often be the medium of transmission of *Salmonella* from animals to man than any other animal food product." Hinshaw and McNeil (1951) in a review of *Salmonella* infection as a food industry problem stress the importance of animal and human reservoirs of the infection, and state that "the genus *Salmonella* is one of the important causes of the infection type of food poisoning."

The isolation and identification of members of the *Salmonella* group from food products present many difficulties. Various methods and modifications, many of which were originally developed for isolating pathogens from feces and sewage, have been proposed throughout the years (Leifson, 1936; Hynes, 1942; Galton and Quam, 1944; Felsenfeld, 1945; McCullough and Byrne, 1952; Ayres, 1953). As a result of these investigations, the use of selective enrichment media is a common procedure and is preferred to direct culture for the isolation of *Salmonella* from suspected materials which contain a large and varied population of microorganisms.

MATERIALS AND METHODS

We have attempted to overcome some of the deficiencies in the recommended procedures by using the dilution-enrichment-subculture method to be described. The primary purpose of the method is to determine the degree of *Salmonella* contamination and the changes in numbers of these organisms during processing and storage of eggs and egg products.

Twenty grams of egg powder are weighed aseptically in a flask containing 180 ml of sterile distilled water and a tablespoonful of glass beads. The egg suspension

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