

YEASTS

A news letter for persons interested in yeasts.

April 1951

Volume I, number 2

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The replies to the questionnaire distributed in Volume I were as follows: 85% favored an informal group in preference to a formal organization; 75% indicated a desire to have the S.A.B. Program Committee arrange for the yeast papers to be given at one session at the May meeting in Chicago; 100% indicated a desire to have an informal meeting after the papers are presented at the session on yeasts; only 50% were interested in the development of a constitution.

Dr. Roger Porter, of the University of Iowa, who is chairman of the Program Committee of the S.A.B., has supplied me with the information below concerning the Chicago meeting:

1. Wednesday afternoon-May 30. Session on Medical Mycology, dealing largely with isolation, properties and infections due to species of Cryptococcus, Dr. Emmons presiding.
2. Wednesday evening-May 30. Symposium entitled "Advances in Mycological Diagnosis" in conjunction with the Chicago Medical Mycological Society, Dr. E. E. Visser convener; Dr. Kligman and Dr. Saslaw participating.
3. Thursday morning-May 31. Session on "Yeasts and Molds," Dr. Wickerham presiding. Meeting for all persons interested in yeasts, Dr. Hedrick presiding, following the presentation of papers.

It is hoped that all of you who attend the meeting in Chicago will be present at these sessions, and particularly the meeting following the papers on "Yeasts and Molds".

2.

In order to have some basis for the continuation of this News Letter, a Plan of Operation has been written and is presented below. This plan has been approved by associate editors Mrak and Etchells.

Plan of Operation

In order to disseminate information to persons working with yeasts and to stimulate interest in research and teaching with yeasts, it is proposed that YEASTS be published and distributed twice per academic year, as for instance in December and April.

The United States shall be divided into three regions: (a) the Eastern-the Atlantic seaboard and Alleghany Mountain states; (b) the Western-the Pacific Coastal and Rocky Mountain states; and (c) the Central-the states not included in (a) and (b) above. Each region is to elect an editor every third year. The editor shall serve three years. The first two years he shall be an associate editor and be responsible for soliciting information from the persons in his region and assisting the editor in questions of policy; during the last year of his term he shall be active editor of YEASTS. This person shall be chosen the year in which his regional predecessor is completing his term as active editor. The editorship shall be rotated among the three regions as follows: Central States, 1950-51; Western States, 1951-52; Eastern States, 1952-53; Central States, 1953-54, etc., until this Plan of Operation is changed by a majority of those persons voting.

Approval or disapproval of this Plan will be considered at the May meeting. Those who will not be present should indicate their desire by writing to the editor or the associate editors. Nominations for an editor from the Central States should be made in writing before the meeting or orally at the meeting.

Although it is proposed that the editorship be shifted from one institution to another after each year, it becomes apparent that for those individuals who are active editors and work for the Federal Government some mechanism to defray the cost of duplication and mailing must be developed. Originally, it was hoped that the institution in question could bear the cost of this service for one year. Consideration of this problem will be one of the topics on the agenda for the meeting in Chicago. Several people who received the first issue of YEASTS expressed a willingness to help defray the cost. The approximate cost of this issue is \$25. Donations of One Dollar will be appreciated. Any excess beyond cost will be forwarded to the active editor for next year, Dr. Mrak.

As previously stated, this News Letter developed out of some conversations among some people interested in yeasts at the meeting of the S.A.B. in Baltimore and the I.F.T. in Chicago. My hours contributed to this venture have been enjoyable ones, and I hope that if this medium aids in communication and inspiration among workers in yeasts its publication will be continued.

Leslie R. Hedrick
Editor, 1950-51

I. RESEARCH IN YEASTS.Carlsberg Laboratories, Copenhagen, Denmark - O. Winge

Continuing their study of yeast cytology and genetics. Forthcoming paper to disagree with Subramaniam and colleagues regarding their interpretations of so-called tetraploid and octoploid yeasts. Some recent papers published (in Comptes-rendus du Laboratoire Carlsberg) are:

- A. Skovsted: Induced Camphor Mutations in Yeast, Vol. 24, No. 21, 1948.
- O. Winge and Catherine Roberts: Inheritance of Enzymatic Characters in Yeasts, and the Phenomenon of Long-term Adaptation, Vol. 24, No. 22, 1948.
- Urs Leupold: Die Vererbung von Homothallie und Heterothallie Bei Schizosaccharomyces pombe, V. 24, No. 27.
- O. Winge and Catherine Roberts: The Polymeric Genes for Maltose Fermentation in Yeasts, and Their Mutability, Vol. 25, No. 2, 1950.

North Carolina Experimental Station and U.S.D.A. - J. L. Etchells

An article (by Etchells and Bell) has been accepted by Food Technology on the hydrolysis of pectin by yeasts. A total of 143 cultures, representing 66 species in 15 genera, were investigated by use of a simplified screening technique to determine their ability to hydrolyze pectin.

The work of the laboratory on the types of yeasts found in northern cucumber brines during a three-year period (1948-1950) is being prepared for publication by Etchells, Costilow and Bell. It is intended that the results of this work will be given at the S.A.B. meeting in Chicago in May.

The article on yeasts in southern cucumber brines appears in the current issue of Farlowia. Representative cultures of each species are on hand in Dr. Wickerham's collection at the N.R.R.L., U. S. D. A., Peoria, Illinois.

Work is in progress on the separation of yeasts from the acid-forming bacteria in fermenting cucumber brines, using antibiotics.

Northern Regional Research Laboratories, Peoria, Illinois - L. J. Wickerham

Work on taxonomy of yeasts and industrial applications of yeasts is continuing. A research position available at N.R.R.L. is described on page 9 of this issue of YEASTS.

Illinois Institute of Technology - L. R. Hedrick

Rosemarie Meyer and Martin Gerber are studying the physiology and nutrition of the genus Kloeckera in an attempt to determine more specific taxonomic characteristics for the species of the genus.

John Bona in his work on the nutrition of Candida has determined that all the members of the genus require added vitamins except C. krusei and C. sorbosa.

Michael Kossoy is studying the metabolic products formed by some of the members of the genus Candida.

Edward Sie is investigating the ability of selected yeasts to produce specific amino-acid decarboxylases.

Robert Betz is determining the minimum vitamin requirements, under various circumstances, for certain members of the genus Hansenula.

Victor Louchious is investigating the nitrogen requirements of the genus Hansenula.

John Gilkison is investigating optimum conditions for producing fat by yeast from corn sugar molasses; determination of types of fat formed by different yeasts, also.

Southern Illinois University, Carbondale - Carl C. Lindegren

Conducting polyploidy in yeast, analysis of asci with more than 4 spores, cytology of copulation and meiosis, galactose, and chromosome mapping.

University of California, Division of Food Technology, Berkeley
4 - E. M. Wraak and M. A. Joslyn

Activities have slowed down to some extent because of the draft picture. However, projects are still quite active. Jack Recca, a graduate student, is still pursuing the problem of the occurrence of yeasts in citrus products, with a view of learning about the types of organisms that occur, and the conditions under which they grow.

Demosthenes Pappiagnis has been studying the fermentation of sucrose by Zygosaccharomyces. It is fairly well known that certain strains of these organisms will show no fermentation of sucrose whatsoever for periods as long as 14 to 20 days, and then all of a sudden display a very rapid fermentation of this sugar.

A third project still under way by Martin Miller concerns the occurrence of yeasts in dried fruit beetles invading the Calimyrna figs.

A project about to be initiated by Tom Nakayama is to be concerned with factors influencing spore formation by certain strains of imperfect yeasts. About a year ago, Phaff observed that certain of these organisms formed a yellow spore under certain environmental conditions, pink under another, and red under a third set of conditions. It is planned to study the nature of the pigments and the particular factors influencing their development.

Miss Nora Neilson, assistant professor of dairying at the University of British Columbia, is completing her thesis on the effect of oxygen on the growth, respiratory and fermentative activities of yeasts. She has succeeded in training a strain of flor yeast to grow continuously in the absence of oxygen and she has some interesting data on the effect of conditions of growth on the respiratory and fermentative activity of this and other strains of yeast.

Donald Vosti is working on the chemistry of yeast autolysis to determine the conditions under which autolysis occurs and the mechanism of autolysis of yeast.

William Moses is actively engaged in the investigation of the apparent equivalence of Vitamin B₁ and Vitamin B₆ for the growth of certain strains of yeast and will study the mechanism of this equivalence in regard to the respiratory and fermentative enzymes secreted by this strain of yeast.

Joseph Tabachnick has just completed his thesis on the "Chemistry and Physiology of Ester Production by *Hansenula anomala*". This work was initiated some three years ago, and while it was in progress two reports along similar lines appeared. Our work confirms the fact that ethyl acetate, the sole ester produced by *Hansenula*, is produced as a result of the respiration of ethyl alcohol, and not as a result of the reversal of esterase activity. Neither in growing cultures, cell-suspensions, or cell-free preparations is ethyl acetate produced from acetaldehyde, pyruvic acid, or acetic acid.

With growing cultures of *H. anomala* and glucose as carbon source, it was shown that ethyl acetate, the ester produced by this organism was formed as a result of an aerobic utilization of ethanol accumulated in the fermentation of glucose. The ester, in turn, was rapidly utilized under aerobic conditions.

The same high yields (19-23% on the basis of carbon content) of ethyl acetate were obtained regardless of the type of nitrogen source added. However, in a nutritionally complex glucose medium, which favored a high growth rate, the maximum yield of ester was obtained within 8 days, while in a medium in which ammonium salts were used as sole nitrogen source as long as 50 days were required.

Using cell suspensions with 2.3% ethanol as substrate and .1 M phosphate buffer, the optimum pH for ester accumulation was found to be between 2.1 and 2.6. The optimum temperature was found to be about 20° C. The high yields of ethyl acetate accumulating at low pH values was shown to be the result of the inhibition of esterase activity of the cells under these conditions.

At pH 2.1, acetic acid was found to be toxic at concentrations of from .01 M to .05 M. At pH 6.7, on the other hand, cell suspensions produced 70 mM of acetate per liter from ethanol

without apparent toxic effect and could oxidize added acetate in concentrations as high as .3 M. Thus, the formation ethyl acetate at low pH enables this yeast to carry out an incomplete oxidation of ethanol to acetic acid without the concomitant accumulation of toxic amounts of this acid. Although only trace amounts of ester were formed from ethanol at pH 6.7, with the addition of DFP (diisopropyl fluorophosphate) a specific esterase inhibitor, a significant yield of ester accumulated at this pH, suggesting that ethyl acetate may be formed and immediately hydrolyzed by the cells at pH 6.7.

Formation of esters from propanol, butanol and amyl alcohol could not be detected by the colorimetric microchemical method of Hestrin. These alcohols were toxic in a concentration of about 2%. The higher aliphatic alcohols in concentrations of .1% and .5% were oxidized to their corresponding fatty acids.

From the high yields of ester formed, the experiment with DFP at pH 6.7 and the observation that ester formation is linked to respiration, it is evident that ethyl acetate formation by H. anomala is most probably an energy coupled reaction and is not the result of a reversal of a simple hydrolytic split mediated by an esterase.

Attempts to obtain an active ester synthesizing cell-free preparation were unsuccessful. A major difficulty was the presence of the esterase in the extracts. The probable mechanism for ethyl acetate formation was discussed.

University of Illinois, Food Technology Department - M. Steinberg

A study of fat production by yeasts is being made.

University of Pavia, Genetic Institute - G. E. Magni

Dr. Magni is studying the ploidy of asporogenous yeasts by means of the X-rays survival curves. The carbohydrate non-fermenting asporogenous species are haploid; the carbohydrate fermenting ones seem to be diploid.

Work is under way on mutations from Rhodotorula to Torulopsis species. Some white mutants of Rhodotorula have been obtained.

University of Tennessee, Knoxville - J. O. Mundt

Several members of the Department of Bacteriology are working on a problem in chlorination of yeasts.

University of Texas, Austin - O. B. Williams

Edna Dudgeon is engaged in a study of the yeasts associated with Drosophila. Cultures have been isolated from the intestinal tract of different species of Drosophila, and an effort is being made to see if yeast species are related to Drosophila species.

University of British Columbia, Vancouver, B.C., Canada -
Professor Nora Neilson (Department of Dairying)

The occurrence of Schizosaccharomyces on dried fruits has been reported only occasionally. We have observed that some strains of the genus grow slowly or not at all on the malt agar usually used for isolation of yeasts but do grow readily when the sugar concentration in the medium is increased. For example, a sample of raisins was washed for household use, then drained and left in a closed container at room temperature for a week or two. A considerable number of the colonies which developed on the raisins were found upon microscopic examination to be fission yeasts with the eight-spored asci typical of Schizosaccharomyces octosporus. When the yeasts were streaked on 5 percent malt agar, the first colonies to develop resembled Zygosaccharomyces. After a further week's incubation at 25° C., a few very small colonies of Schizosaccharomyces were observed. When the yeasts were streaked on the above agar plus 35 percent glucose (cerelose), the colonies of Schizosaccharomyces developed much more rapidly.

While the author was working on the yeast flora of dried prunes with Dr. E. M. Mrak at Berkeley two years ago, similar observations were made. Direct plating of the prunes as outlined by Phaff et al (1) yielded only a few yeasts, none of which was a Schizosaccharomyces. When the prunes were placed in a yeast autolysate medium containing 50 percent sucrose, the first yeasts to develop resembled Zygosaccharomyces as might be expected. However, after two to three weeks at room temperature, cells typical of Schizosaccharomyces octosporus were present in large numbers. These cells failed to develop when they were streaked on 5 percent malt agar but grew readily when 20 percent sucrose was added to the medium.

The fact that the majority of the strains of Schizosaccharomyces encountered in these studies retain their sugar requirement when grown in pure culture indicates that they are true osmophilic yeasts.

¹ H. J. Phaff, E. M. Mrak, Ruth Allemann and Rita Whelton, "Microbiology of Prunes during Handling and Drying", Fruit Product Products Jour. and Am. Food Manuf. 25, 140-141, 1946.

II. UNUSUAL CULTURES AVAILABLE FOR DISTRIBUTION.

University of Pavia, Genetic Institute - G. E. Magni

White mutant of Rhodotorula	<u>mucilaginos</u>	not yet studied for
" " " "	<u>bronchialis</u>	nutritional
" " " "	<u>carbonei</u>	deficiencies

III. NEW TECHNIQUES OR METHODS.

Southern Illinois University, Carbondale - C. C. Lindegren

Improvement of the Hestrin fermentometer.

Department of Agriculture, Science Service, Division of Bacteriology and Dairy Research, Ottawa, Canada - A. G. Lochhead

For the official control of the grade of honey known as "pasteurized", the specifications for which require freedom from sugar-tolerant yeasts, this laboratory has been using a 2.5% agar plating medium containing 60% (60 gm./100 ml.) honey and added yeast nutrients. Dilutions of the test samples are made in sterile 50% honey blanks to avoid plasmoptysis of cells. Plates are incubated at 25° C. for 7 days and then examined for colonies of sugar-tolerant, or osmophilic, yeasts. The medium prevents the growth of "ordinary" yeasts incapable of fermenting honey.

Northern Regional Research Laboratories, Peoria, Illinois - L. J. Wickerham

Four media developed especially for the classification of yeasts are now available from the Difco Company. All are in the form of chemically defined powders. Yeast Morphology Medium consists of trace elements, vitamins, salts, nitrogen, and carbon sources. Yeast Nitrogen Base contains no carbon source or agar and is used for nitrogen assimilation tests. The Vitamin Free Base is used to determine vitamin requirements of yeasts. These media were developed by L. J. Wickerham and co-workers at the N.R.R.L. A leaflet giving the composition of the four media, and describing their use, may be obtained from Mr. H. W. Schoenlein, Director of Bacteriological Laboratories, Difco Laboratories, 920 Henry Street, Detroit 1, Michigan.

IV. NEWS ITEMS, PERSONAL OR OTHERWISE.

Illinois Institute of Technology, Chicago - L. R. Hedrick

In February, the teaching of a course in Identification of Microorganisms was completed, with thirty students registered. About 1/2 of the course was devoted to the study of yeasts.

North Carolina Experimental Station and U.S.D.A. - J. L. Etchells

Abstracts of several articles on yeasts associated with

cucumber fermentations appear in the mimeographed publication AIC-183, obtainable from Box 5578, Raleigh, N. C. Students in particular might be interested in the above publication, which summarizes the cooperative (U.S.D.A.-N.C. Agr. Expt. Sta.) investigations from 1938-1950 on brining and pickling of cucumbers and other vegetables.

Northern Regional Research Laboratories, Peoria, Illinois - L. J. Wickerham.

Dr. Wickerham invites all readers to send him reprints, or manuscripts of papers that have been accepted for publication, which deal with taxonomy, genetics, ecology, or techniques for classification, as he has agreed to write a chapter on yeast taxonomy for the Annual Review of Microbiology, 1952.
University of California, Division of Food Technology, Berkeley 4 - E. M. Mrak

Dr. El Tabey Shehata has completed his work at Berkeley on his Ph. D. thesis entitled; "The Occurrence and Distribution of Yeasts in Drosophila" and is now enroute to his home in Egypt. He will be associated with the Food Technology Division, College of Agriculture, Farouk I, University in Alexandria. It is his hope to continue working on some aspects of yeasts at this institution, although the emphasis of his work will be on food microbiology.

Dr. H. J. Phaff of our Division is on his way home from several months in Delft, Holland. During his time in Europe he found an opportunity to visit with Dr. Winge in Copenhagen, and some of the laboratories in Paris. He is very enthusiastic about the yeast work going on in Holland and Denmark, and he will probably be able to write a brief summary for the next issue of YEASTS. (news item submitted February 20)

Dr. Mrak recently visited Cambridge, and found that Dr. Ingrahm of the Low Temperature Station has been doing a great deal of work on yeasts causing spoilage of juices and concentrates. He is interested in the types of organisms causing such spoilage, and also the conditions under which they grow. He has already published on the effect of SO₂ in citrus juices on yeasts.

Dr. Joslyn is on Sabbatical Leave, and will spend his time in Israel, France and England. At present (written February 20) he is in Israel, and is in close contact with Dr. Leibowitz and his group, who have done so much work on the direct fermentation of maltose.

The course entitled "Biology of Yeasts" is being given again this semester and the enrollment is greater than ever, being 30 students.

University of Massachusetts, Amherst, Mass. - C. R. Fellers

In connection with the Department of Food Technology, a supporting course in "Yeasts and Molds with Industrial

Applications" is being offered in the Botany Department by Dr. Walter M. Banfield. The need for such a course has long been felt, especially to supplement bacteriology. Many graduate bacteriologists and food technologists have had no training in molds and yeasts, a serious omission from many college curricula.

Southern Illinois University, Carbondale - C. C. Lindegren

Dr. Shlomo Hestrin of the Hebrew University in Jerusalem is spending three months in this laboratory collaborating on problems of enzymology.

V. POSITION AVAILABLE.

Northern Regional Research Laboratory, Peoria, Illinois -
L. J. Wickerham

The N.R.R.L., which has one of the largest culture collections of potentially useful yeasts, molds, and bacteria in the United States, is trying to find a Bacteriologist having an elementary knowledge of yeasts for a position on their staff.

The work will involve the classification of yeasts and the development of new industrial processes using species having special characteristics, under the direct supervision of Dr. L. J. Wickerham.

The starting salary is \$3,100 per annum with periodic promotions of \$125 each year up to \$3,850. This position offers excellent opportunities for professional development and advancement to higher positions for someone who has the ability and the initiative to progress in the fields of industrial application or taxonomy of yeasts.

Anyone interested in applying for this position should write or visit the Personnel Office of the Northern Regional Research Laboratory at 825 N. University Street, Peoria, Illinois.

VI. SLIDES AVAILABLE.

University of California, Division of Viticulture, Davis, Cal.-
J.J.B. Castor

Dr. Castor has a collection of fifty photomicrographs of the vegetative cells and spores of various genera and species of yeasts which he has had made into slides for teaching purposes.

If yeast workers would like to have some of these for reference and teaching, he would be willing to loan the basic negatives for reproduction as slides or prints for such purposes.

A list, by number and genus and species, follows:

<u>Photo Number</u>	<u>Genus and Species</u>
157-7	<i>Pichia kluyveri</i>
158-8	<i>Kloeckera austriaca</i>
159-10	<i>Saccharomyces pyriformis</i>
161-15	<i>Pichia chodati</i>
163-17	<i>Endomycopsis Lindneri</i> var. <i>fibuliger</i>
165-19	<i>Debaryo. tyrocola</i>
166-20	<i>Hansenula saturnus</i>
167-23	<i>Hanseniaspora melligeri</i>
168-24	<i>Nectaromyces alpinus</i>
169-28	<i>Hansenula anomala</i>
170-36	<i>Mycoderma La Farii</i>
171-37	<i>Mycoderma chevalieri</i> (Guill)
173-53	<i>Schizosaccharomyces ostosporus</i>
174-40	<i>Mycoderma vini</i> (Will)
175-39	<i>Sacch. cerevis.</i> (Lindegren's H'Z'gStR) (meaning Homozygous Stra
176-41	<i>Mycoderma valida</i> (Liberle)
177-42	<i>Mycoderma cerevisiae</i> (Desm.)
179-46	<i>Rhodotorula rubris</i>
180-51	<i>Sacch. cer. var. ellipsoideus</i> (B)
181-56	<i>Debaryo. membranafasciens</i>
183-74	<i>Sacch. cer. var. ellips.</i> (Str. T)
187-99	<i>Torulopsis Kefir</i>
188-106	<i>Sacch. fragilis</i>
189-112	<i>Schizoblastosporion S-H</i> (Cif)
190-113	<i>Schwanniomyces occidentalis</i>
191-114	<i>Saccharomycodes ludwigii</i>
192-130	<i>Torulaspora fermentati</i>
193-131	<i>Candida albicans</i>
194-179	<i>Brett. bruxellensis</i>
195-186	<i>Kloeckera Lindneri</i>
196-227	<i>Schizosaccharomyces pombe</i>
197-285	<i>Oospora lactis</i> --- ?
198-296	<i>Trichosporon giganteum</i>
199-423	<i>Nematospora nagpurii</i>
200-426	<i>Nematospora phaseoli</i>
205-ss53	<i>Schizosacch. octosp.</i> SPORES
206-ss2	<i>Zygosaccharomyces</i> SPORES
209-ss7	<i>Pichia Kluyveri</i> SPORES
211-ss13	<i>Sacch. tubiformis</i> (P-CN1 ?) SPORES
215-ss20	<i>Hansenula saturnus</i> SPORES
216-ss23	<i>Hanseniaspora melligeri</i> SPORES
218-ss39	<i>Sacch. cerevisiae</i> (Lindegren)
219-ss51	<i>Sacch. cer. var. ellips.</i> (Str. B)
220-ss56	<i>Debaryo. membranafasciens</i> SPORES
226-ssl06	<i>Sacch. fragilis</i> SPORES
232-426a	<i>Nematospora phaseoli</i> SPORES
232-426b	<i>Nematospora phaseoli</i> SPORES

* * * * *

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