YEASTS

A news letter for persons interested in yeasts.

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When the present editor took over it was planned to distribute the first issue in November and the second in April or May. However, responses to calls for news were so slow that the first number was delayed at least three months. Therefore, it is requested that material for the second number be in the editor's hands by April 1st.

Cost of Operation

Funds on hand to cover the costs of mineographing and mailing are meager. Therefore, it would be most helpful if each of those sufficiently interested, would care to contribute a quarter to the kitty.

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I. RESEARCH IN YEASTS:

University of California, Division of Enology, J. G. B. Castor.

We have recently completed a study of yeast spoilage of bottled table wines, chiefly white wines, produced by a maker of top quality California wines. The spoilage which occurred in 100 percent of all lots bottled during late 1950 and early 1951 amounted to the formation of enough granular or flaky sediment, consisting entirely of yeast cells, to make the wines unmarketable. Pure cultures of the "spoilage yeast" appeared to be of a type distinct from the fermenting yeasts deliberately used, and from the wide variety of yeasts in the natural flora found airborne in the winery.

The winery technologists were unable to account for the source of the spoilage, but believed it originated in airborne contamination at bottling. Experimental work eliminated airborne contamination as an important factor and pointed to poor pre-bottling filtration practice. Following minimal improvement in filtration only 20 percent of the lots bottled in the next two months showed any yeast spoilage.

In addition, Mr. G. N. Baldwin has finished work on a Master's thesis problem, during which he studied the basic effects of sulfur dioxide on yeast multiplication. In the course of the work a large discrepancy was found between viable yeast cell counts made by the plating and hanging drop methods. Strains of the genera Saccharomyces, Pichia and Kloeckera were used. The plating method gave variable results even with complex media containing the salts of Williams' medium, glucose, peptone, yeast extract and liver extract. The hanging drop method with the same media in liquid form gave a higher count of viable cells and more consistent results. This contrast was particularly noticeable when suspensions of yeast spores were observed for per cent germination by another student, Mr. J. L. Ricard. Plating of suspensions of Schizosaccharomyces spores liberated from the asci and containing around 100 spores per ml. gave only 5 to 10 per cent of the expected colonies, while in a hanging drop as much as 85 per cent of the spores germinated and gave rise to multiplying vegetative cells. It would be interesting to hear some explanations for this behavior difference between solid and liquid media of the same basic composition. Also to see some concerted work done on providing a satisfactory plating medium and technique for determination of the viable cell count in a suspension of yeast.

Monsanto Chemical Company, St. Louis, Missouri, John G. Kleyn.

The primary objective of our work has revolved about the development of a complex reproducible medium for yeast sporulation. A medium

of this type was developed which afforded 90 to 95 percent sporulation in 4 or 5 days. The strain of yeast used for this work was a diploid strain of Saccharomyces cerevisiae obtained by mating two haploid strains of Saccharomyces cerevisiae which were of opposite mating types (A and alpha). The haploid mating types were obtained from Dr. Gertrude Lindegren. Later work yielded similar results with Saccharomyces globosum and Saccharomyces carlsbergensis var. polymorphus. These yeasts were obtained from Dr. Wickerham.

A synthetic sporulation medium was also developed. This medium, however, did not afford as high a yield (only 50 to 60 per cent sporulation) as the complex medium.

Further studies were concerned with the effect of various environmental factors on sporulation. Some of them were the effect of hydrogen ion concentration, relative humidity, the effect of varying the concentrations of various chemicals in the medium, and the effect of various amino acids on sporulation.

Farouk I University, Alexandria, Egypt, A. El-Tabey.

Microbiological studies have been made on "El-Bouzza", which is a cheap, national, alcoholic drink made from certain type of wheat bread. In 1947, biochemical studies showed that content of sterol (vitamin D) increased to a very great extent during the preparation of El-Bouzza. At present, an effort is being made to isolate the responsible organisms for saccharification of starch, fermentation, and sterol production.

Samples of Egyptian dates of various varieties (Hayani, Samani, Ourabi, etc.) were collected from the markets of Alexandria. Yeast isolations were made and identification of the various isolates is being completed.

Twenty-four samples of yeast isolated from Drosophila in Brazil were obtained from da Cunha, and identification is on the way in the writer's laboratory in Egypt.

University of California, Division of Food Technology, Berkeley, E. M. Mrak.

Tom Nakayama has started work on his M.S. degree on a problem concerned with the nature of the yellow carotenoid pigments in a group of imperfect yeasts whose taxonomic position is uncertain and between Rhodotorula and Cryptococcus. It is hoped that an understanding of the nature of these pigments will aid in their classification.

A paper titled "Yeasts Occurring on Shrimp" by H. J. Phaff, E. M. Mrak and O. B. Williams will soon appear in "Mycologia". Three new species of Rhodotorula, two of Trichosporon and one new variety of this genus are described.

In a cooperative project between Th. Dobzhansky of Columbia University and the yeast workers of the Division of Food Technology, University of California, Berkeley (Phaff, Mrak, Recca, Miller) the collection of yeasts associated with wild species of Drosophila was continued. Three collections were made between July and October of 1951 in the Mather area (4500 ft.), Porcupine Flat area (7000 ft.), and Tioga area (10,000 ft.). One hundred and seventy five cultures were isolated from about 10 species of Drosophila, various slime fluxes and other natural sources. The identification of these yeasts is well under way at present. Some differential attractivity tests were made using a few of the more predominant cultures. (For technique, see Evolution, 5, 97(1951).

Demosthenes Pappagianus has completed his M.S. dissertation titled, "On The Delayed Sucrose Fermentation of Certain Species of Zygosaccharomyces". A summary of his findings follows:

A delayed fermentation of sucrose takes place in 2% sucrose-10% yeast autolysate after 20 to 30 days of incubation at room temperature (23-25°C) by a rather sudden accumulation of gas in the vial of a Durham tube. Several species of Zygosaccharomyces were found to give delayed sucrose fermentation as did Schizosaccharomyces Octoporus. Z. gracilis, one of the yeasts showing delayed fermentation, was used for the ~~remainder of the work.~~ the studies mentioned below.

Delayed fermentation does not appear to be due to mutation or enzymatic adaptation, since the phenomenon cannot be perpetuated by transfer of sucrose fermenting cells. Dried cells grown in sucrose medium apparently have more invertase activity than those grown in glucose; the latter nevertheless do have some activity. Dried or liquid N_2-frozen cells gave a rapid fermentation of sucrose. Raffinose was also fermented by these cells, but more slowly than sucrose.

The rate of inversion of sucrose by dried cells is about the same at pH 4.54 and 5.62 but slightly slower at pH 6.85. The observations on sucrose and raffinose indicate that beta-fructosido-invertase is responsible for hydrolysis of sucrose, though the presence of alpha-glucosido-invertase cannot be excluded.

The presence of glucose had no accelerating effect on sucrose fermentation, but a large inoculum gave a more rapid fermentation of sucrose.

At various pH levels from 3.0 to 7.0, the fermentation of glucose occurred in two to three days, however, the fermentation of sucrose

was not accelerated by any change in pH value, and delayed fermentation occurred only at pH 4.6 and 5.3.

The time for appearance of gas in Durham tubes was shortened when the concentration of sucrose was high. However, even at the highest concentration used, 50% sucrose, gas was not observed for 13 to 15 days.

When cultures were incubated at 37°C, a noticeable amount of gas was produced in 4 to 7 days.

The possible role of autolysis is as yet uncertain.

Martin W. Miller has completed work on his M.S. thesis which was concerned with yeasts associated with Carpophilus hemipterus (dried fruit beetle) in figs. Most of the organisms isolated were Candida krusei and Hanseniospora melligeri. In most cases, the organisms were quite sugar tolerant. Preference tests indicated that the beetle considered C. krusei most desireable. The rate of digestion was rapid, most cells being destroyed in 4-8 hours after feeding.

Jack Recca is completing his M.S. thesis concerned with yeasts in citrus products.

Dr. Phaff, who spent the fall of 1950 in Holland, says that Dr. J. Lodder and Dr. N. J. W. Kreger-Van Rij of Delft, Holland, have prepared a new book on the taxonomy of the yeasts. This volume, which will appear approximately May 1952, is written in English and will cover the perfect as well as the imperfect yeasts. Since the classical works by Stelling-Dekker and Lodder are out of print, the book will undoubtedly be very useful to those interested in taxonomy.

Dr. Leslie R. Hedrick, Department of Biology, Illinois Institute of Technology, Chicago, Illinois.

In an attempt to control the nutrition of Drosophila melanogaster, a survey was made by Sidney Mittler of yeasts that could live on a vitamin and amino acid-free medium. Thus, practically all the nutrition obtained by the flies came from the yeasts. Minimal medium agar is given below:

TABLE I

Vitamin and Amino Acid-Free Medium

Agar	15 gm.	Na Cl	0.5 gm.
$C_6H_{12}O_6$	30 gm.	$Mn\ SO_4$	0.5 gm.
$K\ H_2PO_4$	1 gm.	$Mg\ SO_4$	0.5 gm.
$NaKC_4H_4O_6$	8 gm.	$Fe\ SO_4$	0.5 gm.
$(NH_4)_2SO_4$	2 gm.	H_2O	1000 c.c.
$Ca\ Cl_2$	0.5 gm.		

The following yeasts were not able to live on the above medium:

TABLE II

Ability of Yeasts to Live on Complete Medium and on Vitamin- and Amino Acid-Free Medium.

Yeast	Vitamin and Amino-Free Acid.	M-Y* Complete Medium.
Candida monosa	-	+
C. Pseudotropicalis	-	+
C. mesenterica	-	+
Rhodotorula sanniei	-	+
R. aurantica	-	+
R. suganii	-	+
D. disporus	-	+
D. matruchoti	-	+
D. guilliermondii	-	+
Kloeckera apiculata	-	+
Zygosaccharomyces lactose	-	+
Schizosaccharomyces versatilis	-	+
S. fragilis	-	+
Endomycopsis fibuliger	-	+
Saccharomyces cerevisiae	-	+

*Malt extract-yeast extract medium. Composition given on page 6, U.S.D.A. Bull. No. 1029, 1951.

Those yeasts which grew on the minimal medium are presented in a series decreasing in the ability to aid in the formation of tumors when tu^{50}20 D. melanogaster were reared on them: C. cerevisiae grown on M-Y Medium, 4.7%, Hansenula anomala 4.3%, Pichia membranaefaciens 2.1%, Can-

dida sorbosa 1.9%, Nadsonia fulvescens 1.4%, Debaryomyces globosus 1.3%, Hansenula saturnus 1.2%, Torulopsis utilis 1.0, Rhodotorula gracilis 0.0, R. glutinis 0.0, Geotrichum 0.0.

At present the following medium is used to raise yeasts and fruit flies on vitamin and amino-acid-free medium.

Agar	15 gm.	$MnSO_4$	0.25 gm.
$C_6H_{12}O_6$	10 gm.	$MgSO_4$	0.25 gm.
K_2HPO_4	1 gm.	$FeSO_4$	0.25 gm.
$NaNH_4HPO_4$	2 gm.	H_2O	1000 c.c.
$CaCl_2$	0.25 gm.		

Eduord Sie:- "Determination of Amino Acid Decarboxylases in Yeasts". Definite proof of a specific decarboxylase for glutamic acid in Candida intermedia. This reaction is not the combined deamination and decarboxylation described in Ehrlick as the corresponding amino was demonstrated to be present. There is some evidence that other decarboxylases were present in yeasts. As far as we know, this is the first instance of proof for an amino specific decarboxylase in yeasts.

Studies have been done on "Growth of Yeasts in Olive Residue Hydrolysate" by Elda Tsilenis and Leslie Hedrick.

The olive residue is the waste material after the complete extraction of the olive oil. This waste material at the present time is used largely as fuel. This study is devoted to methods of hydrolyzing this substance and the growing of yeasts cells upon the neutralized digest. After many preliminary experiments the optimum conditions of hydrolyzing the residue were: two successive hydrolyses with 3.5% sulfuric acid at 120°C. to 125°C. for three hours. This method yields 18% to 20% reducing substances calculated as glucose before neutralizing it and about 15% after neutralization.

Yeasts which grew well in this neutralized digest were Torulopsis utitis ATCC 9950, Pichia membranaefaciens ATCC 2254, Geotrichum species I.I.T. 79, Candida krusei NRRL Y1736, and Saccharomyces carlsbergensis ATCC 9080. Those which grew poorly were Candida monosa NRRL Y-1735 and Debaryomyces globosum. Those which did not grow at all were species of the genus Hansenula and four species of the genus Candida.

Nutrients were added to the hydrolysate and studies were made of their effect upon the growth of the yeasts. Among the nutrients tested

were $(NH_4)_2SO_4$, KH_2PO_4, urea, malt extract, and yeast extract in varying quantities and combinations of these.

The 1% malt extract plus 1% yeast extract was found to be the best combination for the T. utilis, P. membranaefaciens, and Geotrichum. The latter organism formed nearly the same growth when 0.1% urea plus 0.5% KH_2PO_4 were used as nutrients. S. carlsbergensis and C. krusei did not improve their yield when additional 1% yeast extract was added to the medium already containing 1% malt extract. Comparative study of their growth and utilization of said medium showed the following:

T. utilis	- 84% sugar used, 950 x 10^6 cells per ml, 28.4% protein formed, based on sugar used.
P. membranaefaciens	- 84% sugar used, 800 x 10^6 cells per ml, 22.7% protein formed, based on sugar used.
Geotrichum	- 82% sugar used, 20% protein formed, based on sugar used.
C. krusei	- 70% sugar used, 470 x 10^6 cells per ml, 25.6% protein formed, based on sugar used.
S. carlsbergensis	- 63% sugar used, 270 x 10^6 cells per ml, 20% protein formed, based on sugar used.

When growth in the hydrolysate was compared with growth in glucose, under similar conditions and when based upon the same consumption of carbohydrate calculated as grucose, the following results were obtained:

T. utilis	- had 11% more growth in the hydrolysate
P. membranaefaciens	- had 14% more growth in the hydrolysate
Geotrichum	- had 7% less growth in the hydrolysate
C. krusei	- formed an equal amount of growth
S. carlsbergensis	- produced 18% more growth

Our results indicate that 4.5% to 5.5% of the olive residue, when calculated on the dry basis, is converted to protein. Therefore, since approximately two million tons of residue is available annually in the Mediterranean Basin, 90,000 tons or 180 million pounds of dry yeast protein may be produced per year.

Persons working on yeasts are: Jules Corbett, Yaye Furutani, Lawrence Klinger, Michael Kossoy, Robert Mamoser, Sidney Mittler, Velma Schmaedter, Edward Sie, Elda Tsilenis, and Clifton Woods.

Department of Bacteriology, University of Illinois, Urbana, Illinois, Dr. S. Spiegelman.

We are presently working on the long term adaptation phenomenon as on the precursors of enzyme formation in the yeasts. We have recently

published a paper in the August issue of the "Proceedings of the National Academy of Sciences" entitled, "A Single Cell Analysis of the Transmission of Enzyme Forming Capacity in Yeast". This paper establishes the particulate nature of the enzyme forming system and estimates the number of particles per cell and the number necessary and sufficient for a cell to form enzyme. We have had a distinguished visiting investigator over a three month period in our Department, Professor R. Y. Stanier of the University of California.

We are very anxious to examine cases of long term adaptation to sugars other than galactose on which we have thus far concentrated. The cultures which are long term adaptors usually are designated as "slow" by the yeast zymologists. It is readily recognized in the following form; when yeasts are tested for carbohydrate fermenting capacity by, let us say, the Durham tube method and a series of tubes are inoculated with uniform inocula, the tubes show no evidence of activity for four or more days and then quite suddenly all or almost all of the tubes simultaneously begin to show evidences of active gas evolution. We are particularly interested in obtaining cultures which are slow on maltose, sucrose, or lactose. Any such cultures which we can obtain will be deeply appreciated.

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

Mr. Calvin C. Kuehner has joined the staff. He will work on the application of yeasts to new industrial processes, and on taxonomy of yeasts. Results of his first project will be submitted to the graduate school of the University of Ohio as his thesis problem for the Ph.D. degree. Mr. Kuehner's family have recently moved from Columbus to Peoria.

A bulletin has been published which presents the concepts of classification on which further taxonomic studies will be based. The reference is:

Lynferd J. Wickerham. 1951 Taxonomy of Yeasts. 1. Techniques of Classification. 2. A Classification of the Genus Hansenula. Technical Bulletin No. 1029, USDA, 56 p., 5 plates. A copy may be obtained by addressing a request to the Northern Regional Research Laboratory at Peoria.

A brief paper will be published in the Journal of Bacteriology early in 1952 announcing the discovery of several previously-undescribed heterothallic yeasts that occur in nature almost entirely in the haploid state. Some named "nonascosporogenous" yeasts are in reality haploid mating types of sporogenous yeasts. For example, strains of Torulopsis sphaerica are mating types of Zygosaccharomyces lactis. Further, Z. lactis strains can easily be converted to nonascosporogenous strains either by the loss of one mating type from the culture, or by loss of the ability

to produce "illegitimate" diploids in a strain originally isolated as a single mating type.

Mating types are available from this Laboratory. To produce ascospores, equal quantities of actively growing mating types are thoroughly mixed in the center of a slant of sporulation medium, then spread over the entire surface of the slant. Spores are formed after 4 to 6 days of incubation at 25°C.

Department of Microbiology and Biological Research, University of Southern Illinois, Carbondale, Illinois, Carl C. and Gertrude Lindegren.

New members of the staff of this department are:

Dr. Dan McClary, who received his Ph.D. from Washington University, St. Louis.

Dr. Leonard A. Sheffner, who received his Ph.D. from the University of Illinois Medical School, Chicago, Illinois.

Professor Norberto Palleroni, A Rotary International Fellow, from the University of Cuyo, Mendoza, Argentina, is studying the genetics of yeast and yeast metabolism.

The following publications from this laboratory have appeared:

Carl C. Lindegren,"The Mechanics of Budding and Copulation in Saccharomyces,"Experimental Cell Research, 2:305-311, September 1951.

A pamphlet has just been published describing "The Manufacture of Dried Food Yeast" and a copy can be obtained by writing to Mr. James D. Veron, Dried Yeasts and Derivatives Dept., Anheuser-Busch, Inc., St. Louis, Missouri.

We have a few fellowships available to graduate students wishing to study genetics, metabolism, or cytology of yeast.

Southern Regional Research Laboratory, New Orleans, Louisanna, Dorothea Teunisson.

We are completing a taxonomic study of yeasts from citrus juices, and commencing a study of the yeasts isolated from stored, rough rice.

American Type Culture Collection, Washington, D.C., Freeman A. Weiss.

We have had numerous requests for Kefir grains to be used in the home preparation of a fermented milk beverage that is believed to have

special therapeutic properties. Yeasts of somewhat doubtful variety and identity are at least components of Kefir grains, and are believed to be either active in the characteristic fermentation or to serve a nutritive role in the multiplication of the lactic bacteria. We are interested in the possibility of lyophilizing the mixture of organisms responsible for the characteristic Kefir fermentation, but thus far have not been able to obtain a starter culture that produces typical Kefir fermentation. We have tried various local sources and have even received two specimens, alleged to be typical Kefir cultures, from Europe, but they have not performed successfully here. We would appreciate hearing from anyone who has Kefir grains or a starter culture that is working properly; in fact, would gratefully accept any information or suggestions on the subject that the clientele of yeasts might care to communicate.

No news from the East this time. Hope all received was included.

E. M. Mrak

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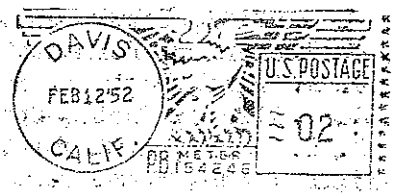

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