Y E A S T S

A News Letter For Persons Interested in Yeast

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The Editor takes pleasure in thanking all those who have contributed to this issue. Without this gratifying support the News Letter cannot fulfill its purpose. The Editors would like to invite others to send in contributions for future issues. It is planned to publish the next issue of the News Letter during the fall of 1955. It would be appreciated if anyone would notify the Editor of additional people in our field who would like to receive the Yeast News Letter.

Cost of Operation. Contributions to help finance the News Letter are voluntary. Present funds on hand are very meager. It would be most helpful if each of those sufficiently interested would contribute a quarter. Many thanks to those who have recently contributed.

The Editors

I. Northern Utilization Research Branch, Peoria, Illinois. Communicated by Dr. L. J. Wickerham.

Dr. Elisa P. Knapp visited the Northern Utilization Research Branch for a few days on her way from the Department of Food Technology of the University of California at Davis, California, where she studied yeasts with Drs. Mrak and Sharf. Dr. Knapp teaches genetics at the University of Sao Paulo, Brazil, and in the future will be yeast taxonomist as well. Her outlook on yeast speciation is very stimulating for it represents the geneticists' viewpoint rather than the opinion of yeast taxonomists in general. Yeast taxonomy could benefit by having a geneticist who is interested in yeasts of all genera from a taxonomic standpoint. We hope Dr. Knapp may be able to devote much of her time at some time in the future to the application of genetics to yeast taxonomy.

Dr. L. J. Wickerham spent nearly two weeks at the University of California at Davis. He presented lectures on taxonomy, sexuality and evolution of yeasts; talked with Dr. Emil Mrak's associates and students on yeast problems, and collected samples for the isolation of microorganisms for the Culture Collection Unit of the Northern Utilization Research Branch.

Dr. J. Lodder plans to visit NURB for three months starting in January, 1956. Concepts of classification, the role of sexuality and genetics in taxonomy, and evolution as a factor in generic diagnosis will be discussed and explored experimentally.

II. Southern Illinois University, Carbondale, Illinois. Communicated by Dr. Carl C. Lindegren and Gertrude Lindegren.

Since the last publication of the YEAST NEWS LETTER, the

following articles have been published or accepted for publication:

Lindegren, Carl C. Non Mendelian segregation in a Single Tetrad of Saccharomyces Ascribed to Gene Conversion. Science 121, 605-607 (April 22, 1955).

Shult, Ernest E. and Lindegren, Carl C. The Hypothesis of Chromosomal Interference. Nature 175, 507 (March 19, 1955).

Lindegren, Carl C. Cultural Variation and Genetics. Chapter 13 INDUSTRIAL FERMENTATION edited by Dr. L. A. Underkofler, Iowa State College. Chemical Publishing Co., Inc., N. Y. pp. 491-517 (1954).

Lindegren, Carl C., McClary, Dan O. and Williams, Marion A. The location of metaphosphate in the yeast cell. Cytologia. In the press.

Shult, Ernest E. and Lindegren, Carl C. The determination of the arrangement of genes from tetrad data. Cytologia. In the press.

Lindegren, Carl C. The identification of fermentative phenotypes in Saccharomyces. Jour. of Bact. In the press.

Pittman, David D. and Pedigo, Paul R. Ultraviolet-induced mutation at the adenine locus in aerobic sufficient cells and in aerobic deficient cells of Saccharomyces. Jour. of Bact. In the press.

Lindegren, Carl C., Lindegren, Gertrude, Drysdale, Robert B., Hughes, John P. and Brenes-Pomales, Arturo. Genetical analysis of the clones from a single tetrad of Saccharomyces showing non-Mendelian segregation. Genetica. In the press.

Pittman, David D. and Pedigo, Paul R. Demonstration by x-ray inactivation of the haploid state of the four clones of yeast derived from an irregularly segregating ascus. Genetica. In the press.

Ogur, Maurice and St. John, Ralph C. Demonstration by cellular nitrogen and turbidity of the haploid state of the four clones of yeast derived from an irregularly segregating locus. Genetica. In the press.

A paper was given on April 30, at the Spring Meeting of the Society of Illinois Bacteriologists at Chicago, entitled "Gene-controlled Sensitivity to Copper in Saccharomyces" by A. Brenes-Pomales, G. Lindegren, and C. C. Lindegren.

III. Bacteriology Division, Science Service, Department of Agriculture, Ottawa, Canada. Communicated by F. G. Lochhead.

Mr. G. B. Landerkin, assisted by Miss C. Darby, has recently completed a re-examination of the vitamin requirements of 18 species of osmophilic yeasts, previously studied in 1942 (A.G. Lochhead and G. B. Landerkin. Nutrilite requirements of osmophilic yeasts. Jour. Bacteriology, 44: 343-351. 1942). In the earlier investigations requirements for biotin, thiamine, pantothenic acid, pyridoxin and inositol were studied. However, in view of discovery, during the past decade, of various new growth factors for microorganisms the following vitamins were employed in addition: riboflavin, niacin, para-aminobenzoic acid, vitamin B_{12}, folic acid and choline. All tests were made in media containing 40 per cent glucose.

The results were surprisingly consistent with those of the earlier work, and indicated that there was no significant alteration in the nutrilite requirements over a period of 12 years. Biotin was essential to all strains. As previously found, the yeasts could be divided into three groups, depending upon whether pantothenic acid was essential, stimulatory, or without effect. With some species inositol, though not essential for growth, was found to be stimulatory.

IV. Department of Microbiology and Public Health, Michigan State College, East Lansing, Michigan. Communicated by Dr. R. N. Costilow.

During the last two years, the foods and fermentations section of the Department has been studying the usefulness of sorbic acid as a selective agent in cucumber fermentations. Extensive laboratory investigations of the effect of this agent on yeasts and acid-forming bacteria common to such fermentations were made and the results

were presented at the Annual Meeting of the Society of American Bacteriologists in New York. This past year, studies were made involving 60 experimental cucumber fermentations under commercial conditions. It is planned to continue this phase of work for at least one more year. The fermentation studies were carried out under a research grant from the Carbide and Carbon Chemicals Company, New York.

Studies are also being made on the identity of yeasts from fresh maple sap.

The following publications involving yeasts have been prepared during the last few months:

1. Costilow, R. N., Ferguson, W. E., and Ray, S. Sorbic acid as a selective agent in cucumber fermentations. I. The effect of sorbic acid on microorganisms associated with cucumber fermentations. Bacteriol. Proc., 1955. Abstract.

2. Sheneman, J. M., and Costilow, R. N. Sorbic acid as a preservative for sweet cucumber pickles. Appl. Microbiol. In press.

V. Massachusetts Institute of Technology, Cambridge, Massachusetts, Department of Food Technology. Communicated by Dr. Cecil G. Dunn.

The Department of Food Technology at the Massachusetts Institute of Technology has recently completed installation of a fully automatic 50 gallon submerged culture pilot plant. It is equipped with an automatic temperature control, a variable speed turbine impellor agitator and provisions for air metering, humidification and sterilization. The main reactor is constructed of stainless steel and can be used under positive head pressure automatically held at pressures up to 30 psig. An automatic pH control is now being installed capable of correcting for either acidic or basic deviations in the same run to within 0.1 pH unit. All controls and valves are operated from an instrument panel.

Doctorate research is now being done on this equipment, on the subject of automatic molasses addition in yeast propagation by Mr. George Fuld under the supervision of Drs. Cecil G. Dunn and Charles N. Frey. The basic project will consist of keeping the sugar level automatically at a constant level throughout the fermentation period, using a modified Bausch & Lomb continuous control refractometer. Possible advantages of the system may be higher net yields, better quality control and a convenient method for determining optimum conditions of a fermentation.

Mr. Wlodzimievz Tereshkovitch is studying oxygenation in submerged fermentation as his doctoral thesis in the Biochemical Engineering subdivision of the Department of Food Technology at M.I.T.

Professor Cecil G. Dunn, Department of Food Technology, M.I.T., was the featured speaker at the symposium on microbiology at the Annual Meeting of the American Society of Brewing Chemists in Philadelphia on May 4, 1955. His subject was "Recent Advances in the Field of Industrial Microbiology and Their Possible Impact on Brewing Technology".

At the Annual Meeting of the American Society of Brewing Chemists, a number of papers of special interest to yeast technologists were presented in addition to the above. Van Engel and Czarnecki (Blatz Brewing Co., Milwaukee) reported on the use of Polymyxin B and other antibiotics in the preparation of pure yeast cultures to be used in a plant propagator. Haas (Liebmann Breweries, Inc., Brooklyn) discussed the influence of antibiotics on biological contaminants in

the brewery. Parsons (Canadian Breweries, Ltd., Toronto) described a program of checking microbiological control in the brewery. Green (Wallerstein Laboratories, New York City) reviewed differential techniques in brewing microbiology. Burger and associates (J. E. Siebel Sons Co., Inc., Chicago) dealt with the microbiology of brewing water and its relation to biological aspects of beer production. It is expected that detailed reports of these presentations will appear in the Proceedings of the A.S.B.C. later in the year.

VI. Department of Biological Control, University of California, Berkeley, California. Communicated by Dr. Edward A. Steinhaus.

1. Limited observations were made on the yeastlike symbiotes of certain Coccidae (Lecanium corni Bouché, Lecanium kunoensis Kuwana, Saissetia oleae (Bern.), and Saissetia nigra (Niet.)). That of L. kunoensis is described for the first time. Attempts were made to cultivate the symbiotes on a variety of artificial media under a variety of conditions, but no definite success was obtained. Contaminating microorganisms (especially Pullularia) commonly confuse the picture and great care must be taken to eliminate these extraneous forms.

2. In many respects the symbiotes of the coccids studied are suggestive of the socalled "black yeasts" of which Pullularia (=Dematium) pullulans (De Bary) is the best known species. This fungus was found to occur on the external surfaces of the coccids and to be abundant in the environment of the insect (i.e., on the twigs and foilage of the host plant). It is not impossible that the symbiotes in the insect have a phylogenetic relationship to the free-living Pullularia,

and that long ago the symbiotic arrangement grew out of the constant association between the insect and the Pullularia in its environment.

3. On a theoretical basis it would appear possible to control certain coccids by altering the biological relationships between them and their symbiotes. It is conceivable that this might be accomplished by altering the insect's environment so as to destroy the symbiote, or, so as to cause the symbiote to become pathogenic for its host or to multiply to a degree that would cause the death of the host. However, attempts to upset the insect-symbiote balance by the use of antibiotics, changes in oxygen tension and hydrogen-ion concentrations, and subjecting the insect to abnormal temperatures, desiccation, and starvation, all failed; at least they failed to do so consistently.

4. Elongated growth forms of the symbiote were observed in a certain percentage of the insects at various ages. It would appear that the occurrence of these elongated forms may not be solely a matter of age, as some workers believe, but at times and to some extent at least, a reflection of the state of the insect's health or vitality. Our observations tend to confirm the idea that the symbiotes of coccids are subject to inhibitory principles which restrain them from excessively (for the host) increasing in numbers and from developing elongated or hyphal growth forms.

VII. Hartley Botanical Laboratories, The University, Liverpool, England.
Communicated by Dr. J. H. Burnett.

Work has continued on the identification of yeasts obtained by crop analysis from Drosophila obscura and D. subobscura, with the assistance of Miss B. Allsopp. Work has also commenced on the

preparation of nuclear autoradiographs of yeast using P^{32} and a short note has been sent to "Nature".

Dr. Burnett wrote the editor that he would be very pleased if any "Saccharomycetologist" visiting England would pay a visit to his laboratory.

VIII. Department of Food Technology, University of California at Davis. Communicated by Dr. H. J. Phaff.

1. Yeast-Drosophila project.

The following paper has been accepted for publication in Mycologia. "Yeasts isolated from Drosophila and from their suspected breeding places in Southern and Central California" by A. M. El Tabey Shehata, E. M. Mrak, and H. J. Phaff. This paper deals with the taxonomic aspects of the yeasts isolated by Shehata in 1949. Due to considerable changes in views on yeast taxonomy in recent years, publication of the taxonomic part of the work (cf. Evolution 6, 325-332, 1952) had been delayed. Three new species of Saccharomyces, all characterized by the possession of kidney-shaped ascospores, have been described. They are Saccharomyces drosophilacibus, S. dobzhanskii and S. phaselosporus.

The following papers have been submitted for publication in Ecology. Studies on the ecology of Drosophila in the Yosemite Region of California. I. The occurrence of species of Drosophila in different life zones and at different seasons by D. M. Cooper and Th. Dobzhansky. II. Yeasts found in the alimentary canal of Drosophila by H. J. Phaff, M. W. Miller, J. A. Recca, M. Shifrine and E. M. Mrak. III. The yeast

flora of the natural breeding sites of some species of Drosophila by H. L. Carson, E. P. Knapp and H. J. Phaff. IV. Differential attraction of species of Drosophila to different species of yeasts by Th. Dobzhansky, D. M. Cooper, H. J. Phaff, E. P. Knapp and H. L. Carson.

We have shown that there is little if any overlapping between the yeasts found in slime fluxes, mushrooms and other breeding places and the yeasts isolated from the alimentary canal of the adult flies. Thus far, it is not known in the region studied, where the yeasts occur naturally that are found in the crops of Drosophila.

The taxonomy of the yeasts isolated will be described in two separate publications, which are still in preparation.

A short note has been published on a method of attracting Drosophila to a banana mash fermented by bakers yeast in which the yeast is subsequently killed by ethylene oxide treatment. The sterilizing agent decomposes on standing for a few days. The reference is "A new method of collecting Drosophila by means of sterile bait" by H. J. Phaff, The American Naturalist, Vol. 89, No. 844, Jan.-Feb., 1955, pp. 53-54.

2. The following paper has been submitted for publication in Mycologia. "Yeasts associated with certain bark beetles" by M. Shifrine and H. J. Phaff.

Of the 169 yeasts isolated five are described as new species. Pichia haplophila does not ferment, assimilates glucose and galactose and forms pellicles on wort and on alcohol medium. An unusual feature is the inability to form pseudomycelium. The spores are hat-shaped.

Rhodotorula crocea. A yellow species of Rhodotorula, starch-like compounds are not synthesized.

Torulopsis nitratophila. Does not [illegible] or ferments glucose very weakly. It assimilates nitrate, glucose and galactose.

Candida silvicola is a nitrate positive yeast, of which over 50 strains were isolated. We were unable to induce sporulation in any of them, not even by mixing strains on sporulation medium. Later Wickerham found mating types for this yeast, which he isolated in an entirely different area of the U.S.A. He is planning to describe the perfect form as Hansenula holstii.

Torulopsis melibiosum is unusual in that it can assimilate melibiose but not raffinose. It also can utilize i-inositol as a single source of carbon, a property not shared by many yeasts.

3. M. Shifrine and H. J. Phaff have recently isolated in pure culture the yeast Saccharomycopsis guttulata, an organism which appears to have resisted living in a test tube until the present time. The natural habitat of this yeast is in the gut of rabbits. It can only grow at temperatures between 35 and 40°C., either aerobically or anaerobically. The cells are extremely short lived on artificial media (a rich yeast autolysate medium plus 2% glucose). The early death of the cells is presumably the reason why other workers have failed to isolate the yeast. Even in a young actively growing liquid medium, only a very small percentage of the cells are able to form colonies on agar. The yeast can ferment several sugars weakly. Details of our work will be published in the Journal of Bacteriology.

4. The following paper will be presented by Dr. E. M. Mrak during the Annual Meeting of the Institute of Food Technologists at Columbus, Ohio in June. "Changes in the yeast flora of olive brines" by E. M. Mrak,

R. H. Vaughn, M. W. Miller, and H. J. Phaff, University of California, Davis, California.

The yeast flora occurring in the brine of green olives during storage in vats and barrels was investigated and 171 yeast isolates were identified. Three stages in floral change were noted:

1. An initial miscellaneous, non-characteristic flora.
2. An intermediate stage in which species of Candida predominated.
3. A final stage in which nearly all isolates were Pichia membranaefaciens.

Relationships of tolerance to salt, pH, and temperature as well as ability of the various groups to utilize lactic and acetic acid were studied.

5. During the recent Annual Meeting of the Society of American Bacteriologists in New York a very successful round table discussion was held in which about 15 persons participated. Problems in medical mycology, general taxonomy of the yeasts, ecology and others were discussed in an informal manner. Dr. Walter J. Nickerson raised the question after the meeting whether it might not be possible to establish a committee on yeast taxonomy, which could meet during the National S.A.B. meetings or at the International Congress of Microbiology and discuss and make recommendations regarding certain difficult and controversial problems in yeast taxonomy.

The editor feels that aside from the difficulty of adequate representation at these meetings, the idea is a very good one and he would like to invite others interested in yeast taxonomy to express their ideas on this matter through the medium of the Yeast News Letter.

6. The following recently published books should be of considerable interest to those teaching or doing research in the field of yeasts.

An introduction to the biology of yeasts by M. Ingram. London, Pitman and Sons, Ltd. 273 pp. 1955.

Dr. Ingram is at the Low Temperature Research Station, University of Cambridge and Department of Scientific and Industrial Research.

Studies on the ecology of yeasts by Aage Lund. Munksgaard, Copenhagen, 1954. 132 pp.