Y E A S T

A News Letter for Persons Interested in Yeast

May 1966 Volume XV, Number 1

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Many thanks to those who have contributed to this issue by sending in news items and accounts of research projects. The next issue will be published in November 1966. A contribution of $1.00 from those who have not contributed for some time would be appreciated to finance future editions of the News Letter. Many thanks to those who have contributed recently.

The Editors

I. Centraalbureau voor Schimmelcultures, Julianalaan 67a, Delft, The Netherlands. Communicated by Miss W. Slooff.

The following new species, for which a description has been published, have been received and placed in the C.B.S. Collection:

Candida aquatica Jones et Slooff CBS 5443
E.B.G. Jones and W. Ch. Slooff, Antonie v. Leeuwenhoek 32, 223, 1966.

Candida curiosa Komagata et Nakase CBS 5688
K. Komagata and T. Nakase, J. Gen. Appl. Microbiol. 11, 255, 1965.

Candida frigida di Menna CBS 5270
M. E. di Menna, Antonie v. Leeuwenhoek 32, 25, 1966.

Candida gelida di Menna CBS 5272
M. E. di Menna, Antonie v. Leeuwenhoek 32, 25, 1966.

Candida incommunis Ohara, Nonomura, et Yamazaki CBS 5604
Y. Ohara, H. Nonomura and T. Yamazaki, J. Gen. Appl. Microbiol. 11, 273, 1965.

Candida nivalis di Menna CBS 5266
M. E. di Menna, Antonie v. Leeuwenhoek 32, 25, 1966.

Candida punica Komagata et Nakase CBS 5689
K. Komagata and T. Nakase, J. Gen. Appl. Microbiol. 11, 255, 1965.

Candida requinyii Szép et Novák CBS 5687
E. Szép and E. K. Novák, Acta botanica Acad. Sci. hung. 9, 447, 1963.

Candida salmonicola Komagata et Nakase CBS 5690
K. Komagata and T. Nakase, J. Gen. Appl. Microbiol. 11, 255, 1965.

Candida soosii Novák CBS 5686
E. K. Novák, Acta microbiol. Acad. Sci. hung. 11, 51, 1964.

Cryptococcus genitalis Castellani CBS 5592
A. Castellani, Dermatologia Tropica 2, 137, 1963.

Lodderomyces elongasporus (Recca et Mrak) v.d. Walt CBS 2605
J. P. van der Walt, Antonie v. Leeuwenhoek 32, 1, 1966.

Pichia acaciae v.d. Walt CBS 5656
J. P. van der Walt, Antonie v. Leeuwenhoek 32, 159, 1966.

Rhodotorula rosa (Nishiwaki) Goto et Yokotsuka CBS 5624
S. Goto and I. Yokotsuka, Bull. Royal Inst. Fermentation, Yamanashi University No. 9, p. 71, 1962 (Japanese).

Rhodotorula zsoltii Galgóczy et Novák CBS 5695
J. Galgóczy and E. K. Novák, Acta microbiol. Acad. Sci. hung. 12, 151, 1965.

Saccharomyces carbajali Ruiz CBS 5313 = NRRL Y-1344
Manuel Ruiz O., An. Inst. Biologia Mexico 9, 49, 1938.

Saccharomyces cidri Legakis CBS 4575
PH. A. Legakis, Diss. Athens 1961 (Greek)

Sterigmatomyces halophilus Fell CBS 4609
J. W. Fell, Antonie v. Leeuwenhoek 32, 99, 1966.

Sterigmatomyces halophilus var. indicus Fell CBS 5628
J. W. Fell, Antonie v. Leeuwenhoek 32, 99, 1966.

Torulopsis anatomiae Zwillenberg CBS 5547
L. O. Zwillenberg, Antonie v. Leeuwenhoek 32, 135, 1966.

Schizosaccharomyces pombe var. acidodevoratus Dittrich CBS 5680
H. H. Dittrich, Zentralbl. Bakt. Parasitenk. II, 118, 406, 1964.

II. Low Temperature Research Station, Cambridge, England. Communicated by Dr. James A. Barnett.

I have described a method of scrutinizing the results of taxonomic growth-tests on different carbon-sources. The object of this scrutiny is to identify the biochemical features that underlie the different responses of yeasts to the growth-tests; and to deduce the routes by which yeasts convert the test-compounds into intermediates of the main metabolic pathways.

The work so far is based on (i) Dr. N.J.W. Kreger-van Rij's thesis on Endomycopsis, Pichia and Debaryomyces and (ii) results of growth-tests on 293 strains of Candida species and 96 strains of Torulopsis species, data of Dr. N. van Uden and his colleagues, most kindly collated by Dr. B. L. Brady.

To extend this analysis, it is necessary to have more data, particularly that of the kind Dr. Brady sent me, i.e. surveys of large groups of strains with large numbers of different substrates. May I ask taxonomists, who have carried out such large scale studies and who would be prepared to cooperate, to write to me?

I expect to be at the International Yeast Symposium in Bratislava in July, and will be glad to discuss this question with anyone interested.

I have published an article on this method: "A biochemical interpretation of some taxonomic differences between yeasts" by J. A. Barnett, Nature, 210, 565 (1966). A note on the subject is also appearing entitled "Biochemical differences between yeasts" by J. A. Barnett, J. gen. Microbiol. (in press).

III. Výskumný ústav vinohradnicky a vinársky, Bratislava, Matúškova ul. č. 21, Czechoslovakia. Communicated by Dr. E. Minarik.

E. Minarik: Ecology of natural species of wine yeasts in Czechoslovakia. Dissertation, Technical University of Agriculture, Brno 1966.

Yeasts and yeast-like microorganisms occurring on grapes, in grape juice and wines in different wine-regions of Czechoslovakia were systematically studied. The dynamics of the evolution and succession of genera and species during the course of fermentation are described. The influence of ecological conditions on the presence and distribution of yeasts (climate and soil factors, temperature and precipitation in vintage years, grape variety, and methods of grape-processing) are discussed.

The association of Kloeckera apiculata - Candida pulcherrima - Saccharomyces cerevisiae var. ellipsoideus is most frequently found in spontaneously fermenting grape juices. The fermentation is initiated by asporogenous yeasts (Kloeckera apiculata, Candida pulcherrima), and the main fermentation by the obligate wine-yeast Saccharomyces cerevisiae var. ellipsoideus. The final stages of fermentations are often carried out by alcohol-tolerant Saccharomyces oviformis, the latter being responsible for re-fermentations of wines with residual sugar.

The representation of yeasts in fermenting pomace of red grape varieties is characterized by a higher proportion of Candida pulcherrima in the microflora. A specific composition of the yeast-flora of grapes could be observed in the wine region of Tokay, where the brewer's bottom yeast Saccharomyces carlsbergensis participates in the alcoholic fermentation of the juice.

The yeast flora of wines consists of relatively few alcohol tolerant and film-forming species. Saccharomyces cerevisiae var. ellipsoideus and Saccharomyces oviformis represent the first, Candida mycoderma and Candida zeylanoides accompanied by Pichia membranaefaciens and Hansenula anomala the second group of yeasts.

The proportion of asporogenous film-forming yeasts in white wines is usually higher than that of red ones. Alcohol tolerance of strains isolated from wines is generally higher than that from grapes and fermenting juice. The most vigorous fermentation activity could be detected in Saccharomyces oviformis with a maximum alcohol production of 19-20 per cent by volume.

The quantitative representation of asporogenous and sporogenous yeast species in the yeast flora of grapes and fermenting juice is affected first of all by climate and weather in individual vintage years. No direct influence of soil conditions and grape-variety could be established.

IV. Institute of Marine Science, University of Miami, Rickenbacker Causeway, Miami, Florida 33149. Communicated by Professor Samuel P. Meyers.

Dr. Samuel P. Meyers is one of 10 participants from the United States invited to participate in the first U. S.-Japan Seminar in Marine Microbiology to be held in Tokyo, Japan, August 15-19. Dr. Meyers will discuss his research and developments in marine mycology, dealing largely with the activities of the marine Ascomycetes and Deuteromycetes. A total of 10 microbiologists from Japan will participate in this invitational seminar, covering a wide range of research areas within the marine microbiological discipline. Following the seminar, the group will participate in the concurrent Pacific Science Congress and tour various laboratories in Tokyo, Osaka and other areas in Japan. Professors K. Tubaki and Arasaki in Japan will be on the program with Dr. Meyers dealing with the biology of marine fungi.

From Japan Dr. Meyers will visit various research laboratories in Europe and will participate in the Seventh Symposium on Marine Biology to be held at Helgoland, Germany, 26-29 September. Dr. Meyers (with Bruce E. Hopper, Nematologist, Canada Department of Agriculture, Ottawa, Canada) will present a paper entitled "Studies on Marine/Fungal Nematode Associations and Plant Degradation." At Helgoland, I will work with Dr. W. Gunkel for a short period in conjunction with his studies of the marine yeasts of the North Sea.

As Editor of the Aquatic Microbiology Newsletter of the American Society for Microbiology, Dr. Meyers would like to hear from microbiologists engaged

in areas of research related to the aquatic (marine, fresh water or pollution) areas. A limited number of the last issue of the Newsletter are available. In the very near future, a list of workers with research specialty will be prepared for distribution to the aquatic microbiologists receiving the Newsletter.

The following four papers dealing with marine yeasts and antiyeast activity have been published from our laboratories:

Ahearn, D. G. and F. J. Roth, Jr. Physiology and Ecology of Psychrotrophic Carotenogenic Yeasts. Dev. in Indust. Microbiol. 7: 301-309, 1966.

Fell, J. W. Sterigmatomyces, a new fungal genus from marine areas. Antonie van Leeuwenhoek 32: 99-104, 1966.

Buck, J. D. and S. P. Meyers. Growth and phosphate requirement of Pseudomonas piscicida and related antiyeast pseudomonads. Bull. Mar. Sci. 16: 93-101, 1966.

Buck, J. D. and S. P. Meyers. In vitro inhibition of Rhodotorula minuta by a variant of the marine bacterium, Pseudomonas piscicida. Helgol. Wiss. Meeresunters. 13 (in press), 1966.

V. Louisiana State University, Department of Botany and Plant Pathology, Baton Rouge, Louisiana 70803. Communicated by Professor J. B. Sinclair.

The following is an abstract of a recently published paper on Geotrichum candidum in Phytopathology 55, 1210-1212, 1965.

Geotrichum candidum: Plant and Animal Isolates Pathogenic to Certain Plant Tisues

Zeineb M. El-Tobshy and J. B. Sinclair

Nine plant isolates of Geotrichum candidum Lk. emend. Carmichael from either citrus fruits or soil, and 12 isolates from animals, were tested for pathogenicity on seven fruits and vegetables. Animal isolates included 11 from humans and one from tortoise. A range of pathogenicity was found. The nine plant isolates were pathogenic to deep-wound-inoculated fruits of tomato, orange, lemon, grapefruit, cucumber, and carrot root. Squash was resistant to these isolates. Most of the 12 animal isolates were pathogenic to inoculated ripe fruits of tomato, orange, and lemon. A single animal isolate was pathogenic to carrot. Pigment was produced in vitro and in vivo by some animal isolates, but not by plant isolates. Temperature studies with one plant isolate and one human isolate showed the optimum for growth to be 27 C for the plant isolate and 30-31 C for the animal isolate.

VI. Medical College of Virginia, School of Medicine, Department of Medicine, Richmond 19, Virginia. Communicated by Dr. H. Jean Shadomy.

Since July, 1965, Dr. Smith Shadomy and I have been at the Medical College of Virginia. With Dr. John P. Utz, a prominent clinician interested primarily in fungal and virus infections and their therapy, we are developing a Division of Infectious Disease in the Department of Medicine.

The following is the summary of a paper now in press in Mycologia, May-June, 1966.

"Preliminary studies on a hypha-forming mutant of Cryptococcus neoformans"

In the Coward strain of C. neoformans, a stable mutant was found which repeatedly produced true hyphae and verticil-like groups of

blastospores when inoculated into modified Sabouraud's agar by the cut-streak method or when cultured subsurface in pour plates. On the surface of agar this mutant produced yeast-like colonies and had biochemical and mouse pathogenicity characteristics typical of C. neoformans.

Preliminary studies of 34 other clinical isolates of C. neoformans indicated that at least two additional strains have the potential for hyphal production.

Therefore, we believe that mutation leading to hyphal formation does occur rarely in C. neoformans. However, because C. neoformans is rarely cultured under conditions ideal for its immergence this phenomenon is not often seen or correctly interpreted.

We now have 43 isolates of C. neoformans, and are asking that cultures from other laboratories be sent to add to our collection. We are particularly in need of fresh isolates and strains which have been carefully studied by others and particularly those reported upon in the literature.

VII. Institute of Fermentation, Yamanashi University, Kofu, Japan. Communicated by Dr. Shoji Goto.

The following is an abstract of a paper now in press:

Microbiological Studies on Petroleum and Natural Gas. VIII. Determination of Red Yeasts Isolated from Oil-brines and Related Materials. H. Iizuka and S. Goto, Jour. Gen. Appl. Microbiol (Japan), Vol. 12, 1966.

SUMMARY

Determinative studies were carried out with the yeasts isolated from oil-brines and other related materials obtained in oil-fields of Japan. The following species were identified: Sporobolomyces japanica nov. species, 1 strain; Rhodotorula rubra (Demme) Lodder, 25 strains; and Rhodotorula glutinis (Fres.) Harrison var. rufusa new variety, 7 strains. The species found in oil-brines differ from those of soil, and all isolates represented the so-called red yeasts which produce carotenoid pigments.

VIII. Institute of Animal Genetics, West Mains Road, Edinburgh 9, Scotland. Communicated by Dr. Colin H. Clarke.

The following papers have been published recently:

Mutagen specificity among reversions of ultraviolet-induced adenine-1 mutants of Schizosaccharomyces pombe. Genet. Res. 6, 204-212, 1965.

Recombination among U.V.-induced adenine-1 mutants of Schizosaccharomyces pombe. Experientia 21, 582-583, 1965.

Mr. Anwar Nasim will be completing his Ph.D. studies here shortly, and then is to spend a year with Dr. A. P. James at Chalk River, Ontario. His studies have been with the adn-7 (red)→adn-7, adn-(white) forward mutational system, using HNO_2, U.V., EMS, hydroxylamine and nitrosoguanidine as mutagens. Of special interest has been the relationship between complete and mosaic mutations at different survival levels. Mr. Anwar Nasim and I will be attending the Yeast meeting at Bratislava, Czechoslovakia in July.

Current work on S. pombe is with the adn-1, 199→adn$^+$ reverse mutation system, following the time course of appearance of reversion induced by HNO_2, U.V. and nitrosomethylurethane at pH 4.5 and 7.2. We are also looking for mutagen specificity in the adn-9 (adn$^-$his$^-$) system, scoring for adn$^+$his$^+$ full revertants and the two possible types (adn$^-$his$^+$ and adn$^+$his$^-$) of "half-revertants", with different mutagens at a variety of survival levels.

IX. Graduate Research Center of the Southwest, Division of Biology, Dallas, Texas 75230. Communicated by Dr. H. Gutz.

Recently we have found that diploid strains of Schizosaccharomyces pombe can be haploidized by treatment with p-fluorophenylalanine. Markers located on homologous chromosomes segregate only in parental combinations, whereas genes on the non-homologous chromosomes show free recombination. These observations correspond to the results obtained by Pontecorvo, Käfer and others with filamentous fungi. In Schiz. pombe only two linkage groups are known but about 30 further markers are available for which no meiotic linkage has been found (Leupold). We hope that we will be able to establish, with haploidization experiments, the number of chromosomes on which these genes are located.

X. Southern Illinois University, Carbondale, Illinois. Communicated by Professor C. C. Lindegren.

The following articles have been published since the last issue of the Yeast News Letter:

(1) Lindegren, Carl C., Bang, Yong Nyu and Bowers, Jr., Wilbert D. A large internal body in yeast mitochondria. Canadian Journal of Genetics and Cytology 7: 589 (1965).

(2) Lindegren, Carl C. Food Yeast. Chapter 28, Vol. 7, pp 226-228, Developments in Industrial Microbiology. American Institute of Biological Sciences, Washington, D. C. (1966).

(3) Hwang, Y. L., Lindegren, G., Bhattacharjee, J. K. and Lindegren, C. C. A genetical study of the lysine biosynthetic pathway in yeast. Abstract, Bacteriological Proceedings, p. 73 (1966).

Dr. Isamu Kondo, a Japanese professor from the Tokyo Jikei-Kai School of Medicine, is spending a year at the Biological Research Laboratory working with Dr. Lindegren on the study of yeast viruses.

XI. University of Puget Sound, Tacoma, Washington 98416. Communicated by Dr. J. G. Kleyn.

The following is an abstract of a paper presented during the Annual Meeting of the American Association for the Advancement of Science (AAAS) at Berkeley, 26-31 December 1965.

Formation of Dwarf Cells by Yeasts Other Than Saccharomyces carlsbergensis

In an initial communication a phenomenon described as yeast dwarf cell formation was observed in fermentations conducted with Saccharomyces carlsbergensis. Such fermentations were characterized by an approximate doubling in the yeast cell population with an increase in yeast crop dry weight only 10-20% greater than that of related control fermentations. Additional characteristics

included smaller cells with increased vacuolation, a slightly greater fermentation velocity, and a slightly lower fermentation liquor pH.

The results reported herein have shown that all of the Saccharomyces species evaluated except one (S. fragilis) formed dwarf cells. Of six other genera examined (Candida, Debaromyces, Hansenula, Pichia, Schizosaccharomyces, and Saccharomycodes) only one formed dwarf cells (Schizosaccharomyces). Of correlated interest was the finding that dwarf-cell-negative yeasts were either poor maltose fermenters or unable to ferment maltose. Inability to ferment maltose did not preclude cell growth in terms of numbers equal to that observed with control dwarf cell strains. However, cell growth in terms of crop yield was approximately 33% greater for control dwarf cell strains. Possible mechanisms for dwarf cell inhibition are discussed.

Publication:

Interrelationships between yeast cell number, size, and yield, during lager beer fermentation. Kleyn, J. G., and N. L. Vacano. Brewers Digest, May, 1966.

XII. Lehrstuhl für Mikrobiologie und mikrobiologische Abteilung im Institut für Gärungsgewerbe, Seestrasse 13, 1 Berlin 65 (West). Communicated by Prof. Dr. S. Windisch.

Studies on microbiology of "Marzipan" (cf. YEAST NEWS LETTER XIV, No. 1, May 1965, p. 4) have been continued.
(S. Windisch u. I. Neumann: Zur mikrobiologischen Untersuchung von Marzipan. 3. Mitt.: Erfahrungen aus der Betriebskontrolle bei der Marzipanherstellung. Süsswaren 9, 540-546, 1965).

Sometimes Marzipan products show watery spots or turn entirely wet. This phenomenon is caused by lipolytic molds as Aspergilli.
(S. Windisch u. I. Neumann: Über die "Wasserflecken" des Marzipans und ihre Entstehung. Zeitschrift für Lebensmitteluntersuchung und -Forschung 129, 9-16, 1965; S. Windisch und I. Neumann: Vorkommen und Bekämpfung von "Wasserflecken" an Marzipan. Süsswaren 10, 88-92, 1966).

More than seven years of research work about genetical analysis and breeding of new types of bottom fermenting Saccharomyces yeasts for technical purposes as beer production have been summarized in: S. Windisch: Ist eine verbesserung technischer Bierhefen über Erbanalyse und Neuzüchtung möglich ? Monatsschrift für Brauerei 18, 274-281, 1965. In the meantime we have published some new results in breeding technical types of Saccharomyces (S. Windisch, H. Oeser u. U. Steckowski: Versuche zur Neuzüchtung von untergärigen Bierhefen. Monatsschrift für Brauerei 19, 29-33, 1966).

We have found that it may be rather difficult to demonstrate that sweets, candies and other sugar containing products have low numbers of Saccharomyces rouxii. We have developed new methods which allow us to detect minimal numbers of Saccharomyces rouxii cells (S. Windisch u. I. Neumann: Über Vorkommen und Nachweis von Saccharomyces rouxii im Marzipan und Kritische Betrachtungen zur Methodik. Süsswaren 10, 314-318, 1966).

The first symposium concerning "working methods and actual results of technical microbiology" has taken place in the Institut für Gärungsgewerbe

April 18-20, 1966. The papers read during the symposium shall be published soon.

A paper previously reported as "in press" has now been published. "Über zwei neue Pilzarten, Protendomycopsis domschii n.g. n. sp. und Endomyces laibachii n. sp. Beitr. Biol. Pflanzen 41, 337-358, 1965.

XIII. Department of Food Science and Technology, University of California, Davis, California. Communicated by Dr. H. J. Phaff.

Mr. Edward Buecher, Jr., a predoctoral student in our laboratory, is investigating the in vitro growth of Saccharomycopsis guttulata. In 1959, Shifrine and Phaff described a complex medium (yeast autolysate, Proteose Peptone, glucose) which gave a high cell yield; however as many as 40% of the cells are found to be plasmolyzed in liquid cultures. Initial data have shown that a number of complete media containing soybean protein also allows a high cell yield. Attempts to vary the percentage of components in the complex media or to decrease the amount of metabolic wastes in the medium failed to eliminate the high percentage of fast-dying cells. Still culture tubes supplied with various gas ratios of N_2, O_2, and CO_2 has shown that a gas mixture of 76.5% N_2, 20% CO_2, and 3.5% O_2 gives up to 95% healthy cells in heavy yield. These findings are consistant with the results of Riche and Scholer (1961) showing the need of a higher CO_2 concentration for cell growth. Presently a "gas machine" is being assembled which will allow a continuous gas flow of adjustable gas mixture; the most satisfactory mixture probably differs from that used in still cultures in which metabolic CO_2 builds up.

Sporulation and germination experiments are being done using the micromanipulation technique. A better growth environment hopefully will yield better germination results than previously reported. Vegetative cells sporulated on YM medium at 18°C can be kept viable for at least 3 years if frozen.

Isolates have been made by Mr. Buecher from wild, black-tailed jack rabbits (Lepus californicus) in the northern California mountains. Although one strain appeared similar in all aspects to strains isolated from domesticated rabbits, a second strain produced atypical colonies and was dimorphic in cellular morphology (yeast and filament forms). The yeast form was similar to other strains except in colonial morphology; filamentous forms with cross-walls produced constricting cells (blastospores) which either went to the yeast or filament type. Typical spores are formed in both types. Differentiation of the blastospores and determination of the factors involved in the transitions are to be studied. Electron micrographs relative to the biochemical data will be taken.

Professor H. J. Phaff will present a paper at the forthcoming International Yeast Symposium at Bratislava and another one at the International Congress of Microbiology at Moscow.

The paper to be given at Bratislava (jointly with Dr. J.F.T. Spencer) is entitled "The taxonomic position of species of the genera Cryptococcus and Rhodotorula". The paper to be presented at Moscow is summarized below.

The presence of an exo-β-glucanase in yeast

by A. Abd-El Al and H. J. Phaff

After sporulation the ascus walls of some species rapidly lyse, liberating the spores. In others the ascus wall remains intact until the spores germinate.

The enzymatic basis of ascus wall lysis forms the basis of this report. Because of the importance of glucan as a wall component different yeasts were examined for β-glucanase activities. Species whose asci rupture readily (e.g. Saccharomyces fragilis) possessed 5 - 6 times the enzymatic activity towards β1→3 and β1→6 glucans as compared to species as S. cerevisiae. Glucanase activity was present extra- as well as intracellularly.

In a defined glucose-mineral medium, extracellular lytic enzyme production by S. fragilis was optimal after 24 hours of aerobic growth at 30°C. Among a number of glycans tested only β1→3 glucan (laminarin) and β1→6 glucan (pustulan) were hydrolyzed at significant rates (relative rates 11:1, respectively).

The action of the enzyme is optimal at pH 5.5. Reaction products determined chromatographically indicated endwise attack on the two polymers, splitting off glucose units. Results also suggest a single-chain mechanism. Similar results were obtained with enzymes from S. cerevisiae and Hansenula anomala. δ-Gluconolactone competitively inhibits the activity of the enzyme.

Partial purification of the enzyme from the concentrated culture fluid of S. fragilis by DEAE-cellulose column chromatography resulted in a 40-fold increase in specific activity. This purified preparation had approximately the same ratio of activities toward laminarin and pustulan. The significance of the enzyme in cell wall lysis will be discussed.

XIV. Department of Microbiology, The Brewing Industry Research Foundation, Lyttel Hall, Nutfield, Surrey, England. Communicated by Miss F. R. Elliott and Mr. M. Richards.

1. The following is a summary from "Inhibition of Yeast Growth by Streptomycin" by Richards, M., and Elliott, F. R., Nature, 209, 536, 1966.

It has previously been recommended that streptomycin (40 μg/ml) be incorporated in media for the selective isolation of yeasts from a contaminated environment. A survey of yeast strains maintained in the British National Collection has revealed that the growth of strains from several genera is completely inhibited by streptomycin within the concentration range of 20 - 50 μg/ml.

Further research on the inhibition of yeast growth has been directed towards the selection of inhibitory agents, which whilst inhibiting the growth of Sacch. cerevisiae, allow the growth of other Saccharomyces spp. particularly those which commonly occur as brewery contaminants.

2. The macro-morphology of "giant colonies" produced upon wort-gelatine has been re-examined as a means of distinguishing between brewing strains of Sacch. cerevisiae. It has been established that this procedure differentiates between a greater number of strains than methods normally used for this purpose. Furthermore, description of the detailed morphology of these colonies has been simplified by the recognition of six sub-specific groups of similar morphological type.

This work will be submitted for publication to the Journal of the Institute of Brewing, in the near future.

XV. Rutgers - The State University, Institute of Microbiology, New Brunswick, New Jersey 08903. Communicated by Dr. J. O. Lampen.

1. A review on the mechanism of action of the polyenic antifungal antibiotics was presented at the April 1966 symposium of the Society for General Microbiology. The volume is titled "Biochemical studies of antimicrobial drugs."

2. Miss Bland Symington has obtained by UV irradiation a yeast mutant whose formation of invertase is not readily repressed by hexoses or by metabolites such as succinate or acetate. In growing cells about 1 - 1.5% of the protein formed is invertase whether raffinose or fructose is the carbon source. The mutant utilizes mannose very poorly and shows an unusual tendency to flocculate.

3. From the nonrepressible mutant, Dr. Norbert Neumann has isolated pure invertase which proves to contain 50% mannan and 2% glucosamine, both covalently linked to the protein. The properties of the purified enzyme were described at the 1966 Federation meeting (Federation Proc. 25: 588 (1966). The abstract follows below:

Purification and Properties of Yeast Invertase
Norbert P. Neumann and J. Oliver Lampen

Invertase has been purified from several strains of yeast approximately 60-fold. Fractions have been obtained with a specific activity equal to 32,000 μmoles sucrose hydrolyzed per min. per mg. protein at 30°, pH 5.0. The enzyme appears to be a glycoprotein which contains an average of 50% mannan and 3% glucosamine. It is soluble in saturated ammonium sulfate and is not precipitated by heat, TCA or picric acid. The enzyme gives a single band in electrophoresis on cellulose acetate and in acrylamide gel columns. Sedimentation analysis reveals several peaks, all enzymatically active and subject to a dissociation-association equilibrium. The major component has an s of 10 S. The molecular weight of the enzyme is estimated to be about 210,000 and by calculation the protein moiety, 105,000. The mode of attachment of carbohydrate to protein has been studied by examining glycopeptides obtained after proteolytic digestion of the enzyme. The predominant amino acids in the glycopeptides were aspartic acid, serine and threonine. (Supported by a grant from USPHS AI-04572).

4. Dr. Santiago Gascon from the Faculty of Pharmacy, University of Madrid, Spain, and Dr. Susumu Nagasaki from Kochi University, Japan, joined our laboratory for the year. Dr. Gascon is studying the secretion of invertase by yeast protoplasts and the mechanisms for its control in the nonrepressible mutant and the glucose-sensitive parent strain. Dr. Nagasaki is investigating the enzyme required (in addition to β-1,3-glucanase) to form protoplasts from our strains of yeast.

XVI. Department of Microbiology, University of Illinois, 127 Burrill Hall, Urbana, Illinois 61803. Communicated by Professor F. M. Clark.

The following is a progress report by Miss Alice Helm and F. M. Clark on the extracellular polysaccharide produced by Torulopsis melibiosum.

We have examined extracellular polysaccharide material which appears as a heavy capsule around the cells of Torulopsis melibiosum. This material appears

to be closely associated with the cells. Relatively large amounts can be precipitated from the medium in which the cells are grown and approximately 10% more removed by mild alkaline treatment (1 N NaOH). Observation of cells after alkaline treatment under the phase microscope reveals a small amount of capsular material still surrounding the cells.

The precipitated extracellular polysaccharide was dialyzed, hydrolyzed with 1 N H_2SO_4, neutralized with Barium carbonate, filtered and tested for products of hydrolysis. Chromatographically the products were identified as mannose and a small amount of glucuronic acid. Two other fractions, an aldobiuronic acid and an acidic polysaccharide, were present. Using a stronger concentration of acid for hydrolysis the aldobiuronic acid yielded small amounts of mannose and glucuronic acid but was not completely hydrolyzed. The acidic polysaccharide treated with stronger acid was hydrolyzed to mannose, glucuronic acid and an aldobiuronic acid. Tests on the polysaccharide obtained by the alkaline extraction of cells gave the same composition as that precipitated from the medium after the cells were removed.

It is interesting that the extracellular polysaccharide of Lipomyces starkeyi reported in the last Yeast News Letter, and that of Torulopsis melibiosum both contained an aldobiuronic acid and that these two fractions appear to be similar chromatographically and chemically.

XVII. Northern Regional Research Laboratory, U. S. Department of Agriculture, Peoria, Illinois. Communicated by Dr. M. E. Slodki.

The following is an abstract of a recently published paper in the Journal of General Microbiology 42, 381-385, 1966.

Extracellular Polysaccharides and Classification of the Genus Lipomyces
M. E. Slodki and L. J. Wickerham

SUMMARY

Strains classified as Lipomyces lipoferus and L. starkeyi produce specific extracellular acidic heteropolysaccharides. The polymer from L. lipoferus contains mannose and glucuronic acid; the polymer from L. starkeyi contains, in addition, galactose and a trisaccharide. This trisaccharide is composed of mannose and glucuronic acid in the molar ratio 1:2. The difference in polysaccharide composition may be the most significant physiological basis yet discovered for separation of species in the genus Lipomyces.

XVIII. School of Public Health, University of California, Berkeley, California. Communicated by Dr. D. Pappagianis.

The following is an abstract of a recently published article in Sabouraudia, Jour. Internat. Soc. Human and Animal Mycol. Vol. 4, part 4, 250-255, 1966.

Studies on Ethanol Production by Cryptococcus neoformans
D. Pappagianis and Ronnie Marovitz

ABSTRACT

Several strains of Cryptococcus neoformans were found to produce small amounts of ethanol (C_2H_5OH) in glucose-containing media as detected by an enzymatic (alcohol dehydrogenase) and a gas chromatographic method. In glucose yeast extract broth, in which Saccharomyces cerevisiae and Candida albicans

produced greater than half the theoretical yield, C. neoformans produced less than 1% of the theoretical yield. Detection of ethanol in the spinal fluid for diagnosis of cryptococcal meningitis appears to be of limited value, unless there is routine determination of ethanol by one of the 2 methods applied, and because of differing yields of ethanol by differing strains.

XIX. Falstaff Brewing Corporation, 1920 Shenandoah Avenue, St. Louis, Missouri 63104. Communicated by Dr. Cavit Akin.

We presented three papers at the recent American Society of Brewing Chemists Convention in Toronto, Canada, and these will be published in full length in the 1966 Annual Proceedings of the A.S.B.C. The abstracts of these papers are given below.

Yeast Sedimentation in Beer Fermentation - The Effect of Wort Carbohydrates

Cavit Akin and Erik Krabbe

Regular wort carbohydrates contain approximately 10 percent monosaccharides, 48 percent disaccharides, 14 percent trisaccharides, and 28 percent tetra-and-higher-saccharides. Our experiments in a newly developed fermentation-sedimentation study system indicated that variations in the carbohydrate ratios in wort upset the beer fermentation and yeast sedimentation patterns. Worts with a monosaccharide content higher than 10 percent on total carbohydrate basis require extended periods for end fermentation. Also, slow and incomplete sedimentation occurs in high monosaccharide worts. The effect of high monosaccharide wort on yeast behavior is reversible. When the yeast is exposed to regular wort after successive exposures to high monosaccharide wort, it behaves normally. High monosaccharide wort has an adverse effect on yeast sedimentation regardless of the flocculation classification of the yeast determined by the flocculation media methods. It appears that a relation exists between the carbohydrate composition in regular wort and the optimum metabolic requirements of yeast.

Deep Fermentation

Cavit Akin and Erik Krabbe

The fixed capital investment for fermenter construction can be reduced by the use of large diameter deep fermenters. In the development and design of deep fermentation systems, both the chemical engineering and the bioengineering principles should be taken into consideration. One of the questions which should be answered is, "What happens to the fermentation kinetics and to the quality of beer when the deep fermentation is applied?". To answer this question we conducted tests in a 44 feet deep pilot fermenter. We observed that in deep fermentations: (1) yeast sedimentation was normal, (2) fermentation followed the conventional pattern, (3) no fermentation stratification took place, (4) product quality matched the quality of conventionally produced beer.

Fermentation Technology

Erik Krabbe, Cavit Akin, Ed. A. Koziboski and Wm. P. Gregory

The design and construction of a brewery fermentor have hitherto been based on a traditional concept of good fermentation practice. This accepted criterion for design resulted from the experience that good beer has issued from fermentors of this type.

In order to optimize the fermentation process, it is paramount to explore what kind of environment will suit the yeast best for producing the beer which we desire. We have attempted such an evaluation.

In this paper, we review the traditional design in the light of our findings. Also, we present some new thoughts on how to optimize the environment for the fermenting yeast in such a manner that this process condition can be provided at a reasonable cost, and with such an effective control system as to insure a consistently fine product.

XX. Brief News Items.

1. Dr. M. Ingram of the Low Temperature Research Station, Agricultural Research Council, Downing Street, Cambridge, England, writes:

> It has been decided to close this Station at the end of 1967, and divide it into two new Agricultural Research Council laboratories elsewhere - a Food Research Institute (at Colney, near Norwich) and a Meat Research Institute (at Langford, near Bristol). As a result, work on yeasts at L.T.R.S. will be broken up. J. A. Barnett will go to Norwich in the coming autumn, 1966; M. Ingram will go to Bristol in the autumn of 1967.

2. Dr. L. R. Hedrick, Department of Biology, Illinois Institute of Technology, Chicago, 60616, writes:

> I have just returned from the American Society for Microbiology Meetings in Los Angeles - May 1-5, 1966. I presented a paper entitled: Effects of indole acetic acid and its photooxidation product on growth of Hansenula subpelliculosa. Leslie R. Hedrick and Kathleen Killick.
>
> Accepted for publication is a paper entitled: Effect of tryptophan on growth and morphology of Hansenula schneggii cells. Malee Sundhagul and L. R. Hedrick. J. Bacteriol. 1966.

3. "Effective August 1, 1966, Dr. S. F. Conti, formerly of the Department of Microbiology at Dartmouth Medical School, will be professor of microbiology and director of the School of Biological Sciences at the University of Kentucky".

4. Dr. J. W. Fell of the Institute of Marine Science, University of Miami, Miami, Florida, recently completed a 3 month cruise in the Antarctic for the study of yeasts in ocean waters. He will begin a year of postdoctoral work in the laboratory of Dr. H. J. Phaff, University of California, Davis, beginning in August of this year.

5. Dr. Clay Hatfield, Professor of Microbiology, California State Polytechnic College, San Luis Obispo, California, attended the First Central American Congress of Microbiology, December 15-18, 1965, held in San Jose, Costa Rica, where he presented a paper.

XXI. Letters to the Editor.

Dear Sir:

> Laboratory work on the isolation and identification of yeasts from sewage and polluted waters has been discontinued, or using a more euphemistic expression, has been phased out. I would like to take this opportunity to thank all those interested in yeasts who have helped me in identification work for their kind cooperation and forebearance.

It should be noted that while I have retained no cultures, I have retained all work sheets produced during the study of more than 5,000 strains of yeasts. On the basis of this information, and information concerning habitats, I plan to develop a comprehensive summary of the work which has been done to date. This should present a unit of information concerning the yeasts of our environment and I would hope that it will encourage others to search more intensively and extensively for these organisms in the particular environmental niches which they might have under study.

I should like to continue keeping in touch with progress in the field of the study of yeasts through the News Letter's pages.

Sincerely yours,

Wm. Bridge Cooke, Ph.D., In Charge
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