Y E A S T

A News Letter for Persons Interested in Yeast

May 1964 Volume XIII, 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 1964. A contribution of $0.50 from those who have not contributed for some time would be appreciated to finance future editions of the Yeast News Letter. Many thanks to those who have contributed recently.

The Editors

I. Centraalbureau voor Schimmelcultures, Julianalaan 67A, Delft, Holland. Communicated by Miss W. Ch. Slooff.

The following cultures, for which a description has been published, have been received by the C.B.S.:

Candida bogoriensis Deinema
M. H. Deinema, Mededelingen Landbouwhogeschool Wageningen 61:1, 1961.

Candida bogoriensis Deinema var. lipolytica Ruinen
J. Ruinen, Antonie van Leeuwenhoek 29:425, 1963.

Candida diffluens Ruinen
J. Ruinen, Antonie van Leeuwenhoek 29:425, 1963.

Candida foliarum Ruinen
J. Ruinen, Antonie van Leeuwenhoek 29:425, 1963.

Candida silvae Vidal-Leiria et van Uden
M. Vidal-Leiria and N. van Uden, Antonie van Leeuwenhoek 29:261, 1963.

Chlamydozyma pulcherrima Wickerham, sex types
L. J. Wickerham, Mycologia 56, 253, 1964.

Chlamydozyma reukaufii Wickerham, sex types.
L. J. Wickerham, Mycologia 56, 253, 1964.

Endomycopsis fasciculata Batra
L. R. Batra, Amer. J. Botan. 50:481, 1963.

Fabospora phaffii van der Walt
J. P. van der Walt, Antonie van Leeuwenhoek 29:319, 1963.

Pichia saccharophila Sasaki et Yoshida
Y. Sasaki and T. Yoshida, Mem. Fac. Agric. Hokkaido 3:178, 1959.

Saccharomyces nilssoni (Capriotti) Santa Maria var. malacitensis Santa Maria.
J. Santa Maria, Bol. Inst. Nacional Invest. Agron. no. 49, 257, 1963.

Saccharomyces norbensis Santa Maria
J. Santa Maria, Antonie van Leeuwenhoek 29:329, 1963.

Saccharomyces osmophilus Barre et Galzy
P. Barre et P. Galzy, Ann. Inst. Natl. Rech. Agron., Ser. E Ann. Tech. Agric. 9:345, 1960.

Schwanniomyces hominis Batista, Vieira et Coelho.
A. Chaves Batista, J. Rego Vieira y R. Pessoa Coelho, Revista Eucuatoriana de Higiene y Medicina Tropical 16:136, 1959.

Torulopsis salmanticensis Santa Maria
J. Santa Maria, Antonie van Leeuwenhoek 29:329, 1963.

Trichosporon loboi Batista, Campos et Oliveira
Z. Chaves Batista, S.T.C. Campos and S. Oliveira, Instituto de Micologia, Universidade do Recife, Publicacão no. 207, 1963.

Mrs. M. J. W. Kreger-van Rij has resigned from the C.B.S. because of her husband's move to the State University of Groningen. Her new address is: Oosterweg 98E, Haren (Gr.), Holland.

II. United States Department of Agriculture, Northern Utilization Research and Development Division, Peoria, Illinois 61604. Communicated by Dr. L. J. Wickerham.

Lynferd J. Wickerham. 1964. A preliminary report on a perfect family of exclusively protosexual yeasts. Mycologia, 56 (2): 253-266. The new family Chlamydozymaceae consists of species that form a dikaryon or diploid state and return to the haploid state without the formation of ascospores. This cycle is designated as protosexuality. It is considered to be the state of sexual reproduction possessed by the most primitive of microorganisms. The type genus is Chlamydozyma, and the type species is C. reukaufii. All are strongly heterothallic, possess sexual agglutination, and hybridize with other species of the genus. The type species of Chlamydozyma hybridizes with the type species of Metschnikowia. Metschnikowia is the most primitive genus of a phylogenetic line of animal and plant pathogens. It consists of species currently classified in the genera Metschnikowia, Coccidiascus, Nematospora, Ashbya, and Eremothecium.

A paper on a cadaver yeast, Hansenula petersonii, and its close relatives (Hansenula jadinii and Candida utilis) should soon appear in Mycologia (has now appeared, Vol. 56, 398-414, 1964 - Editor). This species is tolerant to embalming fluid and phenol solution in which cadavers are stored. It exists in a nonascosporogenous, fermentative form that mutates in YM broth but not on YM agar to a sporogenous oxidative type. Mutation apparently triggers a change in the ability to produce ascospores, to produce substances on the cell walls that are hydrophobic rather than hydrophilic, and a change from more fermentative metabolism to more oxidative metabolism.

III. Instituto di Patologia Vegetale e Microbiologia Agraria, Universita' di Pisa, Italy. Communicated by Professor O. Verona.

1. PICCI, G. and VERONA, O.
Sulla flora blastomicetica di alcuni animali marini. (On the blastomycetic flora of some marine animals.) La Ricerca Scientifica, Parte II, sez. B, vol. 4, no. 1, 1964, p. 85-96.

Numerous strains of yeasts isolated from fishes and other marine animals from the northern part of the Thyrrenian were determined.

Sporogenous yeasts were infrequent; they were Saccharomyces and mainly Debaryomyces species. Asporogenous yeasts were more common and almost exclusively Candida species. Within this genus, C. parapsilosis and C. guilliermondii and their varieties predominated.

2. Researches on the presence of yeasts in wood pulp are in progress. At the moment, species of Trichosporon appear to prevail, the presence of other species is, however, not excluded even in a lower rate.

Researches relative to the physiology of some species in regard to their particular habitat are also in progress.

3. Publications since the last issue of the Yeast News Letter:

O. Verona and A. Rambelli. Presenza di Lieviti e di Specie Lievitiformi in Lettiere di Bosco. (On the presence of yeasts and yeast-like organisms in leaf litter of Eucalyptus.) Annali della Facolta di Agraria, Vol. XXIII, 1962, pg. 37-46.

Summary - A few species of yeasts and three new species of Saprotaphrina occurring in leaf litters of eucalyptus, chestnut and oak are described.

O. Verona. Sezioni Ultrafini di Trigonopsis variabilis Schachner. (Ultrathin sections of Trigonopsis variabilis Schachner.) Mycopathologia et Mycologia Applicata, 30-VIII-1962, Vol. XVII, pg. 345-350.

Summary - Here are presented and described ultrathin sections of Trigonopsis variabilis, with special regard to the cell wall, to cytoplasmic membrane, to bud scars and to the nucleus and mitochondria.

G. Picci and O. Verona. Su di alcuni fattori che influenzano la morfogenesi di Trigonopsis variabilis Sch. (On some factors influencing the morphogenesis of Tr. variabilis Sch.) Estratto da: Atti Istituto Botanico della Universita Laboratorio Crittogamico Pavia, Serie V, Vol. XX, pg. 2-8, 1962.

Summary - As in previous researches, the influence of the nitrogen nutrition and chiefly of the temperature on the morphogenesis of Trigonopsis variabilis Sch. is again demonstrated.

IV. Laboratoire de Microbiologie & Mycologie, Faculté des Sciences, 16, Quai Claude-Bernard, Lyon 7eme, France. Communicated by Professor J. Boidin.

Since the appearance of the last issue of the Yeast News Letter the following article has appeared:

"Quelques levures camerounaises" by J. Boidin, M. C. Pignal, F. Mermier and M. Arpin, Cahiers de la Maboké, 1(2), 86-101, 1963.

Twenty-nine strains of yeast-like organisms have been isolated and studied in detail. They were classified into 19 known species, of which four were sporogenous. One new species of Candida, C. berthetii, was described. This publication in the natural sciences, issued by the "Laboratoire de Cryptogamie du Muséum d'Histoire Naturelle de Paris", has been established only recently and, for this reason, is little known. The authors would be pleased, therefore, to send reprints to all interested mycologists.

A study dealing with a revision of the genus Pichia is in the last stages of editing. All new information on this genus (manuscripts, reprints, strains, etc.) would be very much appreciated.

V. Muséum National d'Histoire Naturelle, Laboratoire d'Ethologie, Parc Zoologique, Paris XII, France. Communicated by Dr. Henri Saëz.

Below is given a list of publications from this institute, together with brief comments:

Recherches sur la flore levuriforme intestinale de l'enfant sain. Revue de Mycologie, T. 26, F.I, pp. 34-39, 1961.

This work was done at Lyon with young children, ages from 3 months to 3 years, in apparent good health. The stools were collected in a nursery in Lyon where the young children were kept.

The genus Candida predominated. It was possible to isolate Candida albicans from individuals in good health and its presence does not imply any form of mycosis.

(with J. Nouvel) - Différentes souches d'Aspergillus recueillies sur des Oiseaux du Parc zoologique au cours de l'année, 1959. Bulletin du Muséum, T. 33, No. 2, pp. 218-220, 1961.

Quelques cas d'aspergillose aviaire observés au Parc zoologique de Paris. Le parasite et l'hôte.
Annales de Parasitologie Humaine et Comparée, T. 26, no. 1-2, pp. 154-165, 1961.

(with J. Nouvel) - Un cas de candidose pulmonaire du Ragondin (Myopotamus coypus Molina) et autres mycoses observées au Parc zoologique de Paris en 1960.
Bulletin du Muséum, T. 33, no. 3, pp. 346-347, 1961.

Candidiasis due to C. albicans in an adult female specimen of Myopotamus coypus, which arrived five days after death at the laboratory. Massive bilateral pulmonary lesions were found in the form of miliary granulation.

Un champignon souvent isolé dans des prélèvements d'origine animale: l'Aspergillus candidus Link.
Bulletin du Muséum, T. 33, no. 3, pp. 341-345, 1961.

Debaryomyces isolés chez des Mammiferes et des Oiseaux. Recueil de Médecine Vétérinaire, Alfort, T. 139, pp. 51-58, 1963.

The 17 strains isolated were identified as follows:

11 D. kloeckeri
3 D. subglobosus
1 D. nicotinanae
1 D. hansenii
1 D. coudertii nov. sp.

Utilisation de l'alcool par les Levures selon une technique en milieu solide.
Bulletin du Muséum, T. 35, no. 1, pp. 116-119, 1963.

This technique employs sloped tubes which we have already used for several years with good results. It is possible to inoculate, at the same time, a whole collection of carbon sources, of which alcohol is used in a concentration of 5 per cent.

Contribution a l'étude de la mycoflore intestinale des animaux sauvages en captivité. 2-Levures isolées chez 77 jeunes Mammifères. Revue de Mycologie, T. 28, f.1, pp. 52-61, 1963.

This work involved the following animals:

13 dead-born mammals
64 mammals aged from a few hours to 10 days.

VI. National Research Council, Prairie Regional Laboratory, Saskatoon, Sask., Canada. Communicated by Dr. J. F. T. Spencer.

The investigation of the yeast population in the Saskatchewan River near Saskatoon is being continued. Yeasts counts rise from 0.1/ml in the river above the city to 5.3/ml just below it, and fall again to 1./ml a few miles farther downstream. Counts in the sewage lagoon are much higher, but fall from 880/ml in the raw sewage to 40/ml in the effluent. Yeasts isolated so far include several Rhodotorula and Cryptococcus species, Trichosporon, Pichia, Candida and Saccharomyces species. Saccharomyces cerevisiae was frequently isolated, and is thought to be a contribution from the local breweries, bakeries, and from householders who do their own baking.

Publications:

Extracellular glycolipids of Rhodotorula species. The isolation and synthesis of 3-D-hydroxypalmitic and 3-D-hydroxystearic acids. A. P. Tulloch and J. F. T. Spencer. Canadian Journal of Chemistry 42, 830-835, 1964.

Abstract - Several species of the red yeast Rhodotorula produce extracellular glycolipids which consist of a mixture of mannitol and pentitol esters of 3-D-hydroxypalmitic and 3-D-hydroxystearic acids. One molecule of the long chain acid is attached to each polyol molecule and most of the remaining hydroxyl groups including the one on the fatty acid are acetylated.

The structures of galactosyl-lactose and galactobiosyl-lactose produced from lactose by Sporobolomyces singularis. P. A. J. Gorin, J. F. T. Spencer, and H. J. Phaff. Canadian Journal of Chemistry, 1964 (in press).

Sporobolomyces singularis, when grown in a medium containing lactose utilized the glucose portion as a carbon source and produced by transgalactosidation a trisaccharide O-β-D-galactopyranosyl-(1→4)-O-β-D-galactopyranosyl-(1→4)-D-glucose (I) and a tetrasaccharide O-β-D-galactopyranosyl-(1→4)-O-β-D-galactopyranosyl-(1→4)-O-β-D-galactopyranosyl-(1→4)-D-glucose (II) (50% combined yield). Oligosaccharides of higher molecular weight were not detected.

β-Galacto- and β-glucopyranosyl disaccharide synthesis by Sporobolomyces singularis. P. A. J. Gorin, J. F. T. Spencer, and H. J. Phaff. Canadian Journal of Chemistry, 1964 (submitted for publication).

Abstract - Synthesis of β-D-galactopyranosyl disaccharides takes place in growing cultures of Sporobolomyces singularis containing lactose and a sugar acceptor. The products of transgalactosylation using various acceptors arise mainly from substitution of secondary rather than primary hydroxyl groups. The minimal structural requirement for reaction with the acceptor appears to be a hydroxyl vicinal to the substituted hydroxyl group. A mechanism that accounts for the formation of secondary disaccharides is postulated.

The organism also contains a β-glucosidase which transfers O-β-D-glucopyranosyl groups from cellobiose to sugar acceptors. The positions of the glycosidic linkages in the disaccharide products are identical to those formed in the corresponding transgalactosylation.

VII. University of Saskatchewan, Saskatoon, Sask., Canada.

The following is a summary of the doctoral dissertation by Dr. P.S.S. Dawson, Granted April 1964. (Information sent to the Editor by Dr. J. F. T. Spencer)

DYNAMIC ASPECTS OF MICROBIAL GROWTH AND METABOLISM
WITH SPECIAL REFERENCE TO Candida utilis

Recent progress in methods for cultivating microorganisms has indicated that growth and metabolism are largely governed by the cells environment. The possibility of using growth under controlled environmental conditions as a method for examining in vivo metabolism in an organism was investigated, and serves as the subject for the thesis.

The yeast Candida utilis was grown on chemically defined media in a specially designed culture vessel by batch, chemostate and continuously phased methods. The three systems possess unique properties that produce characteristic changes in the growth and metabolism of the cells found in the respective cultures.

The differences in metabolism were observed initially as changes in the intracellular amino-acid pools of the cells. The pools were extracted from small samples of culture, by a rapid method based upon membrane filtration procedures. The method was developed later, in a serial manner, to extract other empirical pools, i.e., lipid, nucleotide and others, and these augmented the scope of the investigations.

Analytical procedures based upon paper, thin layer, and gas liquid chromatography, were used to obtain qualitative and quantitative examinations of the various pool constituents.

The cells were grown on chemically defined media devoid of any preformed organic substrates, other than the single carbon source. In this way, transport and permeation phenomena were avoided so that all pool components extracted from the cells were true metabolic products of the cell.

Systematic examination of cells grown by the three methods on glucose media under conditions of carbon or nitrogen limitation, showed that the composition of the intracellular amino acid pool was characteristic of the environmental conditions, and constant in an unchanging environment. When glucose was replaced by other carbon sources, i.e., ethanol, glycerol, acetate or lactate, analogous changes were observed. Overall, the qualitative and quantitative composition of the pool was found to vary with the medium, the growth rate and the stage of growth of the cells.

Analogous changes were observed later when the other pools were examined, i.e. lipid, nucleotide, 'activated' amino acids, phosphate and other constituents.

Other aspects of cell growth and metabolism were examined in relation to the culture methods used; these included effect of oxygen, changes in the morphology of the cells and production of various specific metabolites.

Several important conclusions were drawn from the results obtained. It was established that whereas the batch growth outlines the potential metabolic range of the organisms and chemostat cultures enables selected states to be closely examined, continuous phased culture enables this to be done on the basis of the cell cycle. In this way, it was demonstrated that the metabolism in a cell is in continual flux throughout the whole of its growth, whatever the rate of that growth. The pattern of metabolism in the cell cycle is characteristic for the environment, and constant when the environment is constant.

The results established the continuous cultivation techniques as important new tools for investigating cell metabolism; the chemostat for studying the culture, and phased culture for investigating the cell cycle. Both methods enable examination of integrated metabolic systems in vivo, to be performed under defined conditions, and if need be, these can be continued indefinitely.

The technique of continuous synchronous culture (i.e., phased culture) developed during the investigations also promises to have practical significance, as it could serve as the basis for a new fermentation technique to harvest cellular metabolites at maximal yield.

The results are discussed and considered in relation to contemporary knowledge and to future work.

VIII. D.S.I. Isletme ve Bakim Dairesi, Z.F.H. Md. Laboratuvari, Ulus-Aukara-Turkey. Communicated by Eng. I. Taysi.

The following is an abstract of a paper, soon to be published, based on work done by the senior author under the guidance of Dr. N. van Uden. The work was supported by the Gulbenkian Foundation, Lisbon, Portugal.

OCCURRENCE AND POPULATION DENSITIES OF YEAST SPECIES IN AN ESTUARINE-MARINE AREA

I. Taysi and N. van Uden

Department of Microbiology, Botanical Institute,
University of Lisbon, Lisbon, Portugal

ABSTRACT

A quantitative yeast speciation survey of two temperate estuaries (rivers Tagus and Sado) and adjacent Atlantic zones revealed a decrease of total yeasts counts and of the number of species with increasing distance from the estuaries. The species common in the estuaries as well as in the adjacent zones belonged to genera (Debaryomyces and Rhodotorula) that are ubiquitous in the seas. The species exclusive to and predominant in the estuaries were Candida intermedia, C. lambica, C. silvicola and Torulopsis candida. It was found that the temperature for optimal growth of these species is about 10°C lower than the optimal range of intestinal yeasts (Candida albicans, C. krusei, C. tropicalis and T. glabrata) that have been found to occur in subtropical and tropical waters. The impact of pollution on marine yeast ecology is explained in terms of primary enrichment with nutrients, primary introduction of a wide range of yeast species and secondary selection through the influence of water temperature and other factors.

IX. City of Portsmouth, College of Technology, Department of Biology, Park Road, Portsmouth, England. Communicated by Dr. E. B. Gareth Jones.

Main research topic: An ecological, physiological and taxonomic investigation of freshwater, brackish and marine fungi growing on submerged wood.

During this investigation Candida aquatica was isolated from water scums at Malham Tarn, Yorkshire (a freshwater lake). A paper on its taxonomy is in the press. Further work on its physiology, especially its tolerance of sodium chloride is under way. C. aquatica is of interest due to its habit of forming aeroplane-like cell complexes.

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

1. The description of two new species of Pichia will appear soon in Antonie van Leeuwenhoek.

Two new species of Pichia isolated from slime fluxes of deciduous trees

H. J. Phaff, M. W. Miller, and J. F. T. Spencer

Two new homothallic species of Pichia, associated with exudates of Populus trichocarpa and Salix sp. have been described. Pichia trehalophila n. sp. was isolated from slime exudates in two widely separated Populus trichocarpa trees and

Pichia salictaria n. sp. was isolated from two exudates of Salix sp. The latter species was previously present as an unidentified strain of Pichia in the culture collection of the Centraal Bureau voor Schimmelcultures and had been isolated from a patient in Germany. The natural habitat of P. salictaria is considered to be in association with willow trees.

2. The description of a new species of Metschnikowia will appear in the September issue of the Journal of Bacteriology.

Metschnikowia kamienskii sp. nov., a yeast associated with brine shrimp

J. F. T. Spencer, H. J. Phaff and N. R. Gardner

A description has been given of Metschnikowia kamienskii sp. nov., four strains of which were isolated from brine shrimp (Artemia salina) collected from a saline lake near Watrous, Saskatchewan, Canada. In addition, the authors obtained from Dr. N. van Uden a fifth strain which was originally isolated by Dr. L. J. Wickerham from sporocysts of the trematode Diplostomum flexicaudum parasitizing the hepatopancreas of a lymnaeid snail found in Lake Douglas, Michigan, in which material the yeast was first observed by Dr. Kathleen Hussey of Columbia University. All strains were identical and formed long club-shaped asci, containing a single needle-shaped spore which was pointed at both ends. M. kamienskii differs in several of its physiologic properties and by the size of its asci from the two previously isolated members of the genus, M. krissii and M. zobellii. A key differentiating the three species is given.

3. M. J. Lewis and H. J. Phaff - Release of Nitrogenous Substances by Brewers' Yeast. 3. Shock excretion of amino acids by brewers' yeast. Journal of Bacteriology 87, No. 6 (June), 1964.

ABSTRACT

When Saccharomyces carlsbergensis (two strains) and S. cerevisiae (one strain) were grown in static culture and the harvested, washed cells were suspended in a solution of glucose, amino acids were suddenly released and then rapidly reabsorbed in a space of about two hours. The phenomenon of amino acid release, which was termed shock excretion, varied in intensity with the strain of yeast and was shown to be dependent on the size of the pool of free amino acids within the cells. Shock excretion was independent of osmotic pressure of the suspending medium, but required the presence of a fermentable sugar. D-galactose and maltose caused shock excretion only when yeast was previously adapted to these sugars. Limiting glucose concentrations prevented reabsorption of amino acids, and a further decrease in glucose concentration also limited excretion. Shock excretion was strikingly reduced when the temperature of the suspending medium was lowered.

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

The following is a brief outline of a recent symposium at Freiburg im Breisgau, organized by Professor H. Marquardt and Dr. F. K. Zimmermann and supported by the German Ministry for Scientific Research.

H. Heslot (Paris) 1. Fine structure of genes - especially adn-5 and adn-9 in Schizosaccharomyces pombe.
2. Genetic control of purine biosynthesis in S. pombe.

U. Leupold (Bern) Polarized interallelic recombination - especially in adn-2 and adn-8 of S. pombe.

G. E. Magni (Pavia) 1. Relationship between point mutations and crossing-over - particularly the thr-4, his-1 and can-r systems in Saccharomyces cerevisiae.

2. The mechanism of mutagenic action of acriflavines - 5-aminoacridine acting as a mutagen during meiosis in S. cerevisiae.

W. Laskowski (Berlin) 1. Recessive lethal mutations - their effect on X-ray sensitivity in S. cerevisiae.

2. Correlation of radiation induced mutation rates with the degree of ploidy. $isva^- \rightarrow isva^+$ mutations with X-rays and U.V. in strains of S. cerevisiae.

H. Fritz-Niggli (Zürich) 1. Differential responses of chemically and radiation-induced mutants of the same gene locus (adn-7) and some hot-spot. Particularly the adn-7 locus in S. pombe.

2. Radiation-induced inhibition of nucleic acid synthesis and mutability in S. pombe.

E. A. Bevan (Oxford) 1. A comparison of the patterns of complementation given by EMS and U.V.-induced adn-7 mutants in Saccharomyces.

2. Mutation and infection of the killer character in yeast. The genetics, infectivity and some properties of the killer factor in S. cerevisiae.

N. Loprieno (Pisa) 1. Hot spot mutants in adn-7. Reverse mutability of several mutants at the same hot-spot in S. pombe.

2. Methionine reversion by different mutagens. Response of a met^- mutant of S. pombe to various mutagens and genetic analysis of revertants.

3. Mutagenic analysis of NMU and derivatives. The $met^- \rightarrow met^+$ system used in S. pombe.

H. Holzer (Freiburg) Regulation of enzyme synthesis in yeast by repression and depression of gene activity. DPN^- requiring enzyme converting glutamic acid to α-Ketoglutamic was studied in S. cerevisiae.

F. K. Zimmermann (Freiburg) Reverse mutation experiments in isoleucine-valine-requiring mutants of Saccharomyces cerevisiae. Full and half-revertants studied in different $isva^-$ mutant strains, using several mutagens. Genetic analysis of revertants.

C. H. Clarke (Edinburgh) 1. Mutant expression delay, including mosaic colonies. The adn-1 reversion system and the adenine forward mutation system in S. pombe.

2. Mutagen specificity - particularly in adn⁻met⁻ strains of S. pombe.

This meeting held in Freiburg from 12th-14th of March was extremely enjoyable, friendly and successful. There was much opportunity for informal discussion and the hospitality provided made this venture in European Yeast Genetics a memorable one.

XII. Istituto di Genetica della Universita, Via del Borghetto, 80, Pisa, Italy. (Director: Prof. D'Amato). Communicated by Dr. Nicola Loprieno.

Present research programs with Schizosaccharomyces pombe are:

(1) Genetic analysis of spontaneous and induced reverse mutations in the strain met⁻4,D 19 h⁻. Preliminary results for spontaneous, UV and HNO_2 induced revertants show that 90,72 and 98 per cent respectively are due to suppressor-mutations.

(2) Analysis of the mutagenic activity of different nitroso compounds and their derivatives: only the compounds that yield diazoalkane are mutagenic; the presence of a methyl group increases the frequency of the reversions; mutagenicity increases with increasing pH during treatment; no estimatable killing effect has been found with strongly mutagenic doses.

(3) Genetic analysis of the spontaneous and induced revertants of 18 ad⁻7 "hot spot" auxotrophs, mapped at the site 407 (U. Leupold): three classes of mutants have been already differentiated, namely, (a) mutants that responds to HNO_2; (b) mutants that respond to HNO_2 and UV; (c) mutants that do not respond to either of the two mutagens. For all of the three classes spontaneous reversions have been found.

Dr. Roberto Guglielminetti and Dr. Enrica Michel are cooperating in the mentioned researches.

XIII. Mutagenesis Research Unit, Institute of Animal Genetics, Edinburgh 9, Scotland. Communicated by Dr. Anwar Nasim.

Mutation studies with Schizosaccharomyces pombe

Nitrous acid induced forward mutations in an Ad-7 strain 407h⁻ which accumulates a red pigment are being studied in Schizosaccharomyces pombe.

Some forward mutations produce double mutants which are Ad-7 with Ad-1, Ad-3, Ad-4, Ad-5 or Ad-9.

Colonies thus produced are either completely white or red and white mosaics with white sectors of various sizes.

With nitrous acid treatment at 30°C pH 4.5 producing a survival range of 100 down to 1%, it has been shown that:

1) The ratio of complete whites to mosaics does not change within the survival range tested.

2) Mosaics of many different kinds with 3/4, 1/2, 1/4, 1/8 and irregular white sectors are produced. In addition to these many striated colonies have also been observed. These mosaic colonies when replated give a mixture of red and white colonies proportional to the size of the original sector.

3) A large number of these mosaic and striated colonies have been tested by replating. However, no sectored colonies are obtained in these replating experiments.

Crosses will now be made to study these white double mutants.

XIV. Department of Bacteriology, Indiana University, Bloomington, Indiana. Communicated by Dr. Thomas D. Brock.

Studies on a β-1,3-Glucanase (β-glucosidase) of Yeast

We are currently studying enzymes which may participate in the conjugation process in Hansenula wingei. Because extensive cell wall hydrolysis occurs during the fusion process, we are looking for enzymes which hydrolyze the basal cell wall structure. We are currently working on a β-1,3-Glucanase. This enzyme is widely distributed in yeasts, and we have found it as an intracellular enzyme in baker's yeast, H. wingei and Sacch. lactis. As a substrate we use purified laminarin, and measure the liberation of glucose or reducing materials. Highly purified enzyme from baker's yeast hydrolyzes laminarin, laminaritriose and p-nitrophenyl-β-glucoside, but not cellobiose or salicin. Laminaritriose is hydrolyzed most rapidly. The enzyme is an exohydrolase, and attacks the ends of the polymer chains, releasing only free glucose. No oligosaccharides are released.

Since β-glucosidase of yeast is usually assayed with p-nitrophenyl-glucoside, it is likely that in many cases the enzyme being studied is really β-1,3-glucanase. In whole cells grown on glucose, essentially all of the β-glucosidase activity can be accounted for as β-glucanase. Both the β-glucanase and β-glucosidase activities are inhibited by glucono-delta-lactone.

The above enzyme is probably only one of a series of enzymes involved in conjugation and budding. Also needed are an endo-hydrolase and a β-1,6-glucanase, which we are currently looking for.

XV. The Research Laboratory, Arthur Guinness Son and Co. Ltd., St. James's Gate, Dublin, Ireland. Communicated by Dr. R. B. Gilliland.

Yeasts were killed by a strain of Acetobacter when both were present in bottled beer. When 5.5×10^5 yeasts and 20.5×10^5 Acetobacter per ml. were added to a beer at bottling then after 3 days the count of viable yeasts had fallen to 0; in a control beer with the same number of yeasts but no Acetobacter the yeast count was 7×10^5 per ml. after 3 days. This action was peculiar to this strain of Acetobacter but many species and genera of yeasts were similarly affected. The anti-yeast action was much reduced in synthetic media and did not occur under aerobic conditions. Acetobacter killed by heat, U.V., chemicals, or by disintegration, were without activity. Investigation of the mechanism of this action is continuing.

R. B. Gilliland and J. P. Lacey, Nature 202, 727, 1964.

XVI. The Research Laboratories of Kirin Brewery Co., Ltd., 17 Namamugi, Tsurumi, Yokohama, Japan. Communicated by Dr. Yasuo Umeda.

The following paper was presented at the 8th Convention of the Institute of Brewing, Australian Section, held at Adelaide on 13-17th, April, 1964.

"Some Observations on the Bottom Yeast: Yasuo Umeda"

This paper is a short review concerning some of the studies carried out by Dr. Y. Yamamoto, Mr. T. Sasahara and Mr. T. Inoue, and involved two topics: one is an interaction between a culture yeast and a very similar yeast (which was reported in "Yeast News Letter", Vol. XII, No. 2) and the other is an analysis of the primary fermentation process by culture yeast alone. In the latter work, they observed that yeast begins to bud synchronously a certain period after exhaustion of oxygen from wort, yeast cell division terminates at the middle stage of fermentation and the content of each carbohydrate (trehalose, glycogen, mannan, glucan) component of yeast changes remarkably during this process. It was postulated that the primary fermentation is a continuous interaction between yeast and its environment. (One table, nine figures).

XVII. Sicks' Rainier Brewing Co., 3100 Airport Way So., Seattle, Washington. Communicated by Dr. J. Kleyn.

We have isolated a large number of beer spoilage yeasts from non-pasteurized bottle beer. They were identified as either Sacch. diastaticus type yeasts or yeasts closely related to Sacch. diastaticus since all of the isolates were oval budding cells able to produce superattenuation in finished beer. Preliminary studies by Dr. L. J. Wickerham with certain ones of these isolations showed that they can be differentiated on the basis of their ability to utilize certain oligosaccharides, on their tolerance to osmotic pressure, and on their ability for autoagglutination. Cultures of any such isolations are available upon request. Additional results related to the above subject were presented in the following communication at the May 1964 annual meeting of the Amer. Soc. of Brewing Chemists in New York City: Saccharomyces diastaticus and the biological stability of non-pasteurized bottle beer. Kleyn, J. G., Vacano, N. L. and Kain, N. To be published in Proc. A.S.B.C. 1964.

Other communications are as follows:

Yeast dwarf cell formation. Kleyn, J. G. Presented at the May 1964 meeting of the Amer. Soc. for Microbiology in Wash., D.C. (in press).

Yeast dwarf cell formation by yeasts other than Sacch. carlsbergensis. Kleyn, J. G. (in press).

The fermentology of yeast dwarf cell formation. Kleyn, J. G. and Vacano, N. L. (in press).

Interrelationships between yeast cell number, size and yield during lager beer fermentation. Kleyn, J. G., and Vacano, N. L. To be presented at the fall meeting (Oct. 23 and 24) of the Master Brewers Association of America, District Northwest, which will be held at the Sheraton Hotel in Portland, Oregon.

We would be most happy to visit with anyone interested in yeast should they at any time have an occasion to come to Seattle.

XVIII. Short News Items.

1. The Editor announces with deep regret the death of Professor Raffaele Ciferri, Director of the Istituto ed Orto Botanico della Universita and the Laboratorio Crittogamico of Pavia, on February 12, 1964.

Professor Ciferri was the author of numerous publications on yeast and a Member of the British Mycological Society, Société Mycologique de France, and European Weed Research Council and he received the following honors: Medaglia d'oro dei benemeriti della Scuola, Cultura ed Arte; Doutor em Medicina Honoris Causa da Universidade do Recife; Membro dell'Accademia dei Georgofili.

2. A new edition of the Catalogue of Cultures (Aug. 1963) of the "National Collection of Yeast Cultures" has been received, which is maintained at the Brewing Industry Research Foundation, Nutfield, Surrey, England.

History of the Collection

When in 1948 the Institute of Brewing assumed responsibility for the yeast cultures which formed a part of the National Collection of Type Cultures, a nucleus of some 200 strains was transferred to the laboratories of the Brewing Industry Research Foundation and this formed the basis of the British National Collection of Yeast Cultures. Since that date the Collection has steadily increased as new cultures have been deposited from various sources in many parts of the world and two editions of the catalogue of strains maintained have been issued, one in January 1954 and the other in May 1956. Between the publication of the second edition and this present catalogue the Collection has expanded from 450 to nearly 900 strains.

3. Carl C. Lindegren, Director of the Biological Research Laboratory, Southern Illinois University, Carbondale, Illinois, writes:

"Carl C. Lindegren has been appointed as a consultant for the Planta Piloto de Ron which is the experimental distillery operated by the Agricultural Experiment Station of the University of Puerto Rico on the campus of Rio Piedras. He works there three months every year on yeast problems involved in the fermentation of rum, in collaboration with Mr. Victor Rodriguez Benitez who is Technical Director of the Pilot Plant. This collaboration has been very fruitful, and the work comes at a very convenient time to escape the hard Illinois winter.

An article on 'The Cytology of Yeasts' by Dr. Dan O. McClary of the Biological Research Laboratory has been published in THE BOTANICAL REVIEW, Vol. 30, No. 2 (April-June, 1964)."

4. Dr. L. R. Batra, Assistant Professor of Botany and Curator of the Fungus Herbarium, The University of Kansas, Lawrence, Kansas, 66045, writes:

"I will be spending a year (Sept. 1, 1964 to Sept. 1, 1965) in India to study mycelial hemiascomycetes associated with insects, especially ambrosia beetles. My address will be c/o Department of Pathology, Panjab Agricultural University, Ludhiana, Panjab, India.

5. Dr. R. E. Kallio, Professor of Microbiology, The State University of Iowa, Iowa City, Iowa, writes:

"We are currently investigating the possibility of using alkanes and other hydrocarbons for yeast assimilation tests. A preliminary note has been published (Markovetz and Kallio, J. Bacterial. 87, 968, 1964). We would appreciate cultures of yeasts which appear to have unusual appetites for hydrocarbons."

5. The following three yeast papers were published at the Carlsberg Laboratorium, Valby, Copenhagen, Denmark:

Ditlevsen, E. and V. Hartelius (Physiological Department, Carlsberg Laboratory, Copenhagen, Denmark): On the abnormal germination of spores of Schizosaccharomyces pombe as a result of X-ray irradiation. Compt. rend. trav. lab. Carlsberg 33 (8): 347-359. Illus. Copenhagen 1963.

Spores of the fission yeast, S. pombe, which are lethally damaged by X-rays are able to germinate and grow to a certain extent. The individual spores were followed in living cultures in Bürker-Türk chambers until no further development occurred (after two or three days' incubation), and their nucleic patterns were observed in stained preparations.

X-irradiated spores were observed to stop growing at various stages of development, but in a majority of them the cell division mechanism seemed to be blocked, whereas cytoplasmic growth was able to continue. This caused the appearance of elongated cells, up to ten times normal length. An elongated cell seemed to produce approximately the same amount of cytoplasm as contained in the progeny of a normal cell which had multiplied during the same time.

The block of cell division was not complete. In many apparently undivided cells mitosis was in progress and in one-third of the cases the development proceeded beyond the point of cell division. The cells underwent cell division although so late that the daughter cells were also elongated. Furthermore, half of the cells divided in an abnormal way by merely forming a cell plate without the daughter cells parting.

Also purely cytoplasmic changes occurred, manifested by abnormal cell shapes in 5% of the cells. Ordinarily this did not affect the mitotic activity.

Aa. Hvidt and J. H. R. Kägi (Chemical Department, Carlsberg Laboratory, Copenhagen, Denmark): Effect of coenzymes on hydrogen-deuterium exchange of yeast alcohol dehydrogenase as determined by infrared spectrophotometry. Appendix: Infrared spectra of reduced and oxidized NAD-coenzymes in D_2O. Compt. rend. trav. lab. Carlsberg 33, 16: 497-534. Illus. Copenhagen 1963.

The deuterium exchange of yeast alcohol dehydrogenase, pD 8, was studied in the presence and absence of the coenzymes nicotinamide-adenine dinucleotide (NAD^+) and dihydronicotinamide-adenine dinucleotide (NADH), using an infrared spectrophotometric method. The exchange was followed by recording the decrease of the amide II

absorption band as a function of time (time-difference spectra) and sample composition (constituent-difference spectra). The evaluation of the absorption data in terms of numbers of exchangeable peptide hydrogens was made on the basis of absorption parameters determined previously for lysozyme.

About 4% of all peptide hydrogen of the protein was protected completely by NAD^+ and partially by NADH from exchanging with the solvent. The effects were interpreted as evidence that specific changes in enzyme conformation occur in response to coenzyme binding. The possible bearing of such changes on the binding sequence of coenzyme and substrate to the apoenzyme is discussed.

In an appendix the infrared spectra of NAD coenzymes in D_2O were recorded in the region between 1350 and 1760 cm^{-1}. From comparison with spectra of adenosine and 1-methyl nicotinamide assignments of the main absorption bands to the different heterocyclic moieties of the coenzymes were made.

Ditlevsen, E. and V. Hartelius (Physiological Department, Carlsberg Laboratory, Copenhagen, Denmark): On the sensitivity of germinating spores of Schizosaccharomyces pombe to X-rays. Compt. rend. trav. lab. Carlsberg 33 (7): 319-346. Illus. Copenhagen 1963.

The employed strain of S. pombe is heterothallic, and when cultivating a mixture of the two mating types on malt-agar, four-spored asci are formed which liberate their spores. Most of the experiments were carried out in Bürker-Türk blood counting chambers with the spores suspended in wort-gelatin. All chambers received the same dose of X-rays, 16000 r. The viable count (a measure of the resistance to X-rays) was defined as the ratio between the number of colonies developed in irradiated chambers and in control chambers.

Resting spores had a viable count of 5-6% and young cells 45-50%, showing the cells to be much more resistant to X-rays than spores, and the aim of the investigation was to follow the development of the X-ray resistance of the spores during their germination into young cells. The development of the spores in the chambers was observed microscopically and at different germinating stages chambers with spores were irradiated.

During the first 12 hours of germination period the resistance of the spores dropped from the initial viable count value of 5-6% to a very low value, 0.2%, whereafter it increased again and at 16-17 hours regained the initial value. The drop in resistance occurred during the initial stage of germination, the swelling. The time when the initial resistance was regained coincided with the inception of cytoplasmic growth, and during the later stages of germination the resistance increased roughly in proportion with the increase of cytoplasmic matter. The point of germination at which the individual spore reached its minimum of resistance was probably only for a short period of its mitotic cycle, presumably during the pro-metaphase.

Many of the lethally damaged spores were able to go through part of the germination cycle, showing signs of blocked division mechanism (elongated growth). This will be further discussed in a following paper. (See page 14, No. 5).