Y E A S T

A News Letter for Persons Interested in Yeast

May 1967 Volume XVI, Number 1

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Because of the Editor's trip to Japan this issue appears somewhat later than is customary. The next issue will be assembled towards the end of November. Although a reminder for copy will be mailed in the fall, news items may be sent in at any time.

H. J. Phaff

I School of Medicine, Division of Dermatology, University of California, Los Angeles, 90024. Communicated by Dr. Frances Keddie.

The communication which follows will be presented at the XIII International Congress for Dermatology, Munich, 1967.

TINEA VERSICOLOR: THE ELECTRON MICROSCOPIC MORPHOLOGY OF THE GENERA MALASSEZIA AND PITYROSPORUM

Frances M. Keddie

The electron microscopic morphology of Malassezia furfur (Robin) Baillon 1889, in the scales of tinea versicolor was reported in 1966 (Keddie). The morphology of the fungus in vitro has now been compared to that of the fungus in the human epidermis and found to be the same. Furthermore, electron microscopic examination of Pityrosporum ovale (Bizzozero) Cast. et Chalmers 1913, Pityrosporum orbiculare Gordon 1951, Pityrosporum canis Gustafson 1954, and the cultures isolated by Panja, in 1927, from the scales of tinea versicolor and from scales of the human scalp show them to have the same definitive type of cell wall construction and vegetative reproduction by budding and fission that characterize Malassezia furfur. Panja stated that he had proved the two genera i.e. Malassezia and Pityrosporum, to be one class of organisms of the same genus. The genus Malassezia having been created first should have preference, hence P. ovale becomes M. ovalis (Bizz.) Panja 1928.

The distinctive characteristics of the genus Malassezia (syn. Pityrosporum) are clearly shown in thin sections at magnifications of 5,000 to 40,000 times. The cell wall appears to be made up of delicate fibrils, sometimes compressed in layers, and sometimes separated from one another in regular sequences which correspond to protrusions into the cytoplasm. These protrusions, as seen in profile in sections examined in serial order, form ridges and furrows on the inner surface of the cell wall. Furthermore the ridges and furrows are slightly oblique to the long axis of the cell. Less frequent are finger-like protrusions of the cytoplasm which form small pits in the substance of the cell wall.

A second distinctive characteristic is the septal wall which serves to separate the bud from the parent cell. In serial sections this wall appears to grow inward from the peripheral wall at the level of the base of the bud. As the wall grows it begins to separate into two layers which mark out the future line of fission. Once the crosswalls are completed, the two cells are forced apart by the continued growth of these crosswalls which now form the walls of the new buds. The outer wall splits more or less completely apart, leaving edges that form a rim around the periphery of the crosswall.

Apart from the cell wall and the septum, these fungi have the usual cytoplasmic structures of other yeasts. The matrix, which is dense, is filled with ribosomes, mitochondria, a nucleus, vesicles of various sizes and complexity, and a well developed reticulum. The folds of the reticulum, which parallel the ridged contours of the cell wall, appear to arise as off-shoots from the nuclear envelope. The mitochondria contain inner membranes which sometimes appear to be lamellae and sometimes tubules.

Old and new isolates were examined. The old ones were those of Panja isolated from the scales of the scalp (MRL 3073) and from tinea versicolor (MRL 3074) which had been maintained in the Mycological Reference Laboratory of the London School of Hygiene and Tropical Medicine since 1930; a strain of tinea flava (MRL 3075) isolated by Dr. E. C. Smith in Lagos in 1928; and the type culture of P. ovale (CBS 1878) isolated by Rhoda Benham in 1939. The recent isolates were strains of P. canis (CBS 1879) (RDSVS 2079, 63a), 4 strains from patients with tinea versicolor (UCLA 6,7,14,186) and 1 from a patient with blepharitis (UCLA 26).

All the cultures examined were grown under the same conditions and prepared for electron microscopic examination at the same time, except for one culture which was allowed to grow until hyphae developed. Aside from the rather consistent differences in shape between the isolates from the human skin and from the ear wax of dogs, there is an added difference in the fact that the animal species does not require lipids for growth in culture. It is more difficult to separate the so-called P. ovale from the so-called P. orbiculare by morphology alone. It seems likely they will prove to be distinct species when they have been studied biochemically. The electron microscopic techniques of preparing thin sections which were examined in serial sequences have shown that the two genera Malassezia and Pityrosporum are alike in their structure and their vegetative reproduction, thus confirming Panja's reports of 1927, 1946, and 1962. Inasmuch as his cultures, though now 40 years old, present the same morphology as he described, we believe his work to be confirmed and concur with his statement that there should be one genus, namely Malassezia, for which Pityrosporum becomes a synonym.

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Panja, G. (1962). Cultivation of the Malassezia species (M. furfur and M. Ovale); their successful isolation, morphology, pathogenicity and classification. Proc. Symp. Sch. trop. Med., Calcutta, 5-6 Feb., 1959. Calcutta. Sch. trop. Med. Pp. 199-212.

II Institute of Fermentation, Yamanashi University, Kitashin-machi, Kofu, Japan. Communicated by Dr. Shóji Goto.

Coprophilous Fungi from KarakorumI.

Junta Sugiyama and Shóji Goto

The Journ. of Japanese Botany, 42 No. 3 75 - 84 1967

Seven species of coprophilous fungi, including one new species, i.e., Sporobolomyces coprophilus and one variety, i.e., Cryptococcus albidus var. ovalis, were reported from goat dung collected in Hispar Valley, Karakorum, Pakistan.

Aspergillus candidus and Cryptococcus albidus show a world-wide distribution on animal dungs.

These fungi, i.e., Stemphylium ilicis, Sporobolomyces coprophilus, Cryptococcus neoformans, Crypt. albidus var. ovalis and Rhodotorula marina, will be added for first record to the dung microflora.

Sporobolomyces coprophilus Sugiyama et Goto

Growth in malt extract: After 3 days at 25°C, cells oval to long oval, 3-5.5 x 6-13 µ, single, in pairs and short chains. Pellicle formed wrinkled, consisting of budding cells, irregularly formed sterigmata, ballistospores and true hyphae. Sterigmata and ballistospores, however, are very rarely formed.

Growth on malt agar: After one month at 17°C, streak culture reddish orange, shiny, moist, smooth and slightly rough. Pseudomycelium, hypha-like sterigmata and ballistospores observed at the margin.

Slide cultures: Primitive pseudomycelium formed.

Sporulation: Ballistospores oval to long oval and rarely discharged.

Fermentation: Absent.

Sugar assimilation: glucose +, galactose -, sucrose +, maltose +, lactose -.

Assimilation of KNO_3: Positive.

Ethanol as a sole source of carbon: Scanty growth.

Splitting of arbutin: Negative.

Iodine reaction of the extracellular polysaccharide: Negative.

III Institut de Recherches Viti-Vinicoles, Matuskova 21, Bratislava, Czechoslovakia. Communicated by Dr. E. Minarik.

The following is a summary of a recently published paper.

Minarik, E. Occurrence of contaminant yeasts and yeast-like microorganisms in wine during bottling. Wein-Wissenschaft, 22, 67-74, 1967

The composition of the yeast flora in natural and sparkling wines during and after bottling from two factories in Czechoslovakia was examined. It could be repeatedly established that film-forming yeasts of the genus Candida get into the wine from vessels and other cellar equipment. The true wine yeast Sacch.cerevisiae var. ellipsoideus is displaced by the more active and resistant Sacch.oviformis during storage of wines. These yeasts, causing refermentations of wines with residual sugar, originate not only from contaminations but also by the multiplication of a few cells originating from grapes.

Film-forming yeasts do not occur in sparkling (champagne) wines during and after the fermentation in the bottle.

Yeasts of the genus Torulopsis and Rhodotorula, occurring as contaminants in the factory and occasionally in the wine, do not actually influence the yeast flora of wines because of their very low alcohol tolerance.

Additional publications: Mikrobiologie des Weines - Research Results

over the years 1956-1960, VITIS 6, 82-88, 1967; Research Results over the years 1961-1964, VITIS, 6, 89-98, 1967. Author E. Minarik.

Ecology of natural wine yeast species in Czechoslovakia by E. Minarik. Biologicke Prace - Edicia Vedeckych Kolegii Pre Vseobecnu A Specialnu Biologiu Slovenskej Akademie Vied XII/4, 1966. (In Czech with Russian, German and English summaries; 106 pages).

IV University of Miami, Institute of Marine Sciences, 1 Rickenbacker Causeway, Miami, Florida 33149. Communicated by Dr. Samuel P. Meyers.

1. Dr. D. G. Ahearn is working this summer with Drs. Meyers and Roth at the University of Miami in a continuation of their cooperative studies on the marine yeasts. Dr. Ahearn would like to note that his home address is as follows:

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Georgia State College
33 Gilmer Street, S. E.
Atlanta, Georgia 30303

2. The following mycological papers from our group have either been published since the last News Letter, or are in press or preparation:

Speciation and densities of yeasts in human urine specimens. Sabouraudia 5: 110-119. 1966.

Urine specimens from 1013 patients were selectively and quantitatively cultured for yeasts. The presence of yeasts was correlated with the age, sex, and physical condition of the individual. Yeasts occurred at an incidence of 17.6% with approximately 6% of the patients demonstrating urine populations of at least 10^5 yeast cells/ml. Candida albicans, Torulopsis glabrata, and C. tropicalis were the most common species isolated. These taxa occurred in high densities most frequently among diabetics and terminal patients. Torulopsis glabrata was the most common species occurring in high numbers in urine specimens from diabetics with urinary tract pathologies; one diabetic patient died of a fungemia due to T. glabrata. The physiological and morphological characteristics employed for the identification of yeasts from urine specimens were critically examined and reviewed.

Mycological investigations of the Black Sea. Bull. Mar. Sci. 17: 000-000. 1967.

A collection of 558 molds and yeasts from the waters of the Black Sea has been characterized and population densities established. Of 174 water samples taken, 84 were positive for yeasts while 97 contained molds. The average yeast density was less than 10 cells/L while densities in excess of 200 cells/L were found in only six samples. The maximal yeast population was 150 cells/L. Decrease in incidence, density, and average number of species of yeasts was noted with increasing depth. Predominant taxa included the yeasts, Debaryomyces hansenii, Candida diddensii, Rhodotorula rubra

and R. glutinis and the hyphomycetes, Cladosporium spp. and Aureobasidium pullulans. In general, low concentration of fungi prevented definitive correlation of individual species distribution with hydrographic aspects.

Distribution of yeasts in the Indian Ocean. Bull. Mar. Sci. 17: 454-470. 1967.

Yeasts from the North Sea. Helgol. Wiss. Meeresunters. 14: 000-000. 1967.

Distribution and taxonomy of yeasts in the Florida Everglades and adjacent waters. In preparation.

Normal fungal flora of the human small and large intestine. In preparation.

Yeasts from the North Sea. II. Physiological and ecological characteristics. In preparation.

Ultrastructure of the ascospores of the genus Hanseniaspora. In preparation. (with N. J. W. Kreger-van Rij)

Dimorphism in the genus Rhodotorula. In preparation. (This paper involves electronphotomicrographic studies of filamentous chlamydospore-bearing strains of Rhodotorula. These yeasts are quite similar to Dr. Banno's reported sexual phase, however, they are clonal isolates and have not yet been shown to possess a conjugate phase).

3. Dr. Meyers has heard from Dr. B. Norkrans, University of Gothenburg, Marine Botanical Institute, Gothenburg SV, Sweden who writes that she is now "fishing" for yeasts in the marine environment. Dr. Norkrans has done considerable work in the field of cellulose decomposition by fungi and it will be quite interesting to follow her line of inquiry with the marine yeasts.

V Laboratorio de Micologia, Facultad de Quimica, Montevideo, Uruguay. Communicated by Dr. R. C. Artagaveytia-Allende.

Uruguay has decided to make a study of the soil and my laboratory will participate in identifying the yeast strains isolated from the soil samples received.

At the same time we are training young people in this subject.

During the last January we were invited to participate in the 1st Latin American Symposium on Soil Microbiology, integrated in the XVIII National Congress of Botany, that took place, in January, in Rio de Janeiro, Brasil.

We gave a paper on "Yeast Taxonomy" and I was chairman of the session "soil as a source of pathogens".

The Symposium was superb because very good papers were presented.

In our laboratory we are doing our usual work and Miss Lucy Teira just finished one project about the presence of yeast in meat. This work is being published in "Atti dell' Istituto Botanico e Laboratorio Crittogamico dell' Universita de Pavia, Italia."

VI Brewing Industry Research Foundation, Nutfield, Surrey, G. Britain. Communicated by Dr. A. H. Cook.

This Foundation, which houses the British National Collection of Yeast Cultures, is inevitably concerned at any one time with a wide variety of yeast studies. Currently these range from necessarily long-term work on the ability of yeasts satisfactorily to withstand lyophilic drying and subsequent storage with regard to both laboratory cultures and larger quantities, to studies on protoplast development and the structure and variability of yeast cell-walls; from studies on levels and fluctuations of specific enzymes to assessments of over-all fermentation performance of Saccharomyces strains. Two recent investigations call, however, for more specific mention since they promise to deal with two long-standing difficulties.

The first relates to the emphasis often placed in commercial brewing on the use of specific strains of S.carlsbergensis or cerevisiae; in the latter case it is moreover quite common to employ yeasts consisting of a mixture of two or even several strains. The reasons for a particular preference are important but so slight, such as small differences in the production of minor fermentation products, that it is almost hopeless to expect that it would be possible to distinguish the desirable from the less desirable strains by orthodox morphological or biochemical examination. Nevertheless it is obviously highly important to have some means of checking, sometimes over long periods, that the strains employed have remained unchanged and in the case of a mixture of strains that the composition of the mixture has remained unchanged. This need seems to be well met by adequate observation of the macroscopic appearance of giant colonies produced under controlled but not necessarily highly specific conditions (M. Richards, J. Inst. Brew., 1967, 73, 162). In broad principle the method is not new and it remains necessary to allow 10 days or preferably 3 - 4 weeks for the development of well-characterised colonies. However, the degree of discrimination which the recent procedure permits seems greater than has previously been appreciated and an analysis of a mixture on a percentage basis can be carried out without recourse to micromanipulation.

The second investigation concerns the related difficulty of recognising at an early stage any contamination of a selected strain or strains of Saccharomyces by a nearly related infacting strain. Recognition of contaminating bacteria, of yeasts of different genera or even of different species presents no great difficulty but hitherto it has not been possible to recognise a low level, perhaps 1 : 10^6, of infection which is then only revealed when the level has risen to give rise to some irregularity in operation. This particular difficulty also seems likely to be met by the use of a serological method (M. Richards and T. W. Cowland, J. Inst. Brew., in the press). In outline the method consists in using a serum containing antibodies produced in rabbits

by a yeast suspension. The serum acquires its usefulness by the combination of the antibodies with the antigenic yeasts. The combination is rendered visible by the use of a fluorescein-conjugated goat anti-rabbit serum whereby the combination becomes brightly fluorescent in ultraviolet light. As so far described the serum will react indiscriminately to cultured and wild yeasts but it is rendered more specific by cross-absorption using a brewing yeast. Moreover, by combining two such sera, a preparation is obtained which is capable of detecting all brewery contaminants of the genus Saccharomyces so far encountered. Furthermore by suitable selection of the brewing strain used for the cross-absorption of the serum and by attention to details of the actual cross-absorption procedure the final serum becomes capable of picking up wild-type S.cerevisiae strains. Observation of slides illuminated by ultraviolet light easily reveals the presence of as few as one 'wild' cell per million 'culture' cells so that the limit would seem to be set by the chance of including any wild yeast present at this low level in any given number of slide preparations.

VII Research Laboratories of Kirin Brewery Co. Ltd. Takasaki, Gunma Pref., Japan. Communicated by Dr. Yoshino Kuroiwa.

Below are abstracts of two papers presented by members of the Microbiology Section of our laboratories and published in "The Report of the Research Laboratories of Kirin Brewery Co., Ltd., No. 9, 1966".

1. FATAL PHENOMENON OF YEAST CAUSED BY ACETIC ACID BACTERIA
Tatsuhiko Kaneko and Yasushi Yamamoto

It was observed that, when yeast was stored in tap water, brewery pitching yeast died sometimes much faster than yeast of the same strain cultured in the laboratory.

Yeast cultured in the laboratory, however, died rapidly when stored in the water which was used for covering brewery pitching yeast, but died very slowly when the water was subjected to sterile filtration. Therefore, the fatal phenomenon of brewery pitching yeast could be attributed to microbial contamination.

Meanwhile, predominant, contaminating bacteria in brewery pitching yeast such as Flavobacterium, Aerobacter and Pseudomonas did not show any yeast-cidal activity when used either alone or mixed in spite of their high population, while, some others had the lethal activity in spite of their extremely small population. All of the latter were identified as acetic acid bacteria.

Being preserved in tap water, yeast produced ethanol at the expense of its reserve glycogen, and the ethanol would be oxidized to acetic acid by the coexisting acetic acid bacteria. The fatal phenomenon of yeast was observed when the concentration of the acid exceeded 0.5%.

2. MORPHOLOGICAL STUDY OF YEAST DURING BREWERY FERMENTATION
Yasushi Yamamoto and Takashi Inoue

Growth of yeast cells during the primary fermentation of brewing was

studied morphologically. Yeast cells began to bud simultaneously after a certain period of the lag phase and proliferated synchronously during the fermentation. The first budding was highly synchronized and the maximum budding index (expressed as : number of budding cells / total number of cells) recorded was 95%. Division of cells was not observed after the second budding, which was less synchronized, with many nutrients left in the fermenting liquor (6-7°Plato). The number of times of budding during the fermentation could be varied by changing pitching rate.

VIII National Research Council, Prairie Regional Laboratory, Saskatoon, Sask., Canada. Communicated by Dr. J. F. T. Spencer and Dr. E. von Rudloff.

A number of yeasts, most of which had originally been isolated from spoiled beer or from similar sources, were tested for their ability to degrade or modify some of the components of hop extract. A medium containing hop extract and yeast extract was inoculated with a 48-72 hr culture of yeast (4% v/v) and incubated on a rotary shaker for seven days, after which the steam-volatile components were recovered and analyzed by gas-liquid chromatography. The yeasts could be divided into three major groups: those which caused little change in the steam-distillable fraction, those which apparently modified one or more components, as determined by the shifting of a major peak in the gas-liquid chromatogram, and those which caused extensive degradation and complete disappearance of the major steam-distillable components of the hop extract medium. Brewer's yeasts belonged to the first group, and many of the spoilage yeasts occurred in the second and third groups.

IX State Institute for Technical Research, Biotechnical Laboratory, Helsinki. Communicated by Dr. T. M. Enari.

At a Scandinavian Symposium on Aroma Research (Fredensborg, Denmark, April 1966) mr. V. Mäkinen from this laboratory read a paper on the formation of aroma compounds during continuous fermentation of beer. Experiments were carried out in a 2 liter laboratory fermenter with ordinary wort as nutrient. Three different temperatures were used: 8°C, 14°C and 20°C and two bottom yeast strains, one very flocculent and one non-flocculent, were compared.

With both yeast strains the amount of fusel alcohols (Komarowsky reaction) was increased with increasing fermentation temperature: 50-55 µg/ml at 8°C; 60-70 µg/ml at 14°C and 90-120 µg/ml at 20°C. The flocculent yeast strain produced more fusel alcohols at high temperatures than the non-flocculent strain.

Gas chromatography showed that optically active amyl alcohol (2-methyl butanol) is produced at a higher rate during continuous fermentation than in batch fermentation. The amount of isoamyl alcohol (3-methyl butanol) in an ordinary lager beer is about five times that of the optically active amyl alcohol. During continuous fermentation at 20°C equal amounts of the two amyl alcohols are produced. Continuously fermented beer also contains more n-propanol than batch fermented beer.

X Department of Agriculture, University of Mie, Kamihama-Cho, Tsu-City, Mie Prefecture, Japan. Communicated by Dr. Morio Akaki.

The following three papers have been published:

1. Studies on the Brewing of Sake using Pure Cultures of Saccharomyces sake instead of "Moto". (I) Production of Yeast Cells by Shaking Culture: R. Miyazaki, O. Nagano, H. Yoshida, and M. Akaki. Jour. Soc. Brewing, Japan, 60 (11), 989-992 (1965).

In order to brew sake on an industrial scale with pure cultures of yeast, conditions of media for the manufacturing of yeast cells were examined in shaking culture. Results as mentioned below were obtained.

1) A medium containing malic acid, urea, and molasses was superior in regard to the yield of yeast, stability of pH, and the efficiency in separating and washing yeast, etc.

2) With this medium the required quantity of yeast could be manufactured by shaking the culture in 5 l conical flasks.

2. Studies on the Brewing of Sake using Pure Cultures of Saccharomyces sake instead of "Moto". (II) Brewing of Sake on an Industrial Scale: R. Miyazaki, O. Nagano, H. Yoshida, and M. Akaki. Jour. Soc. Brewing, Japan, 60 (12), 1103-1106 (1965).

This paper deals with further studies, in which an attempt was made to brew sake on an industrial scale by using pure cultures of sake yeast instead of "moto".

Results obtained were as follows:

1) At the first stage of the "moromi mash" made with a pure culture of sake yeast, the yeast propagated a little more vigorously, and a characteristic was recognized in its components as compared with the ordinary mash. But the mash with a pure culture of the yeast showed similar changes to those in the ordinary process after the middle stages of brewing, and sake was brewed safely.

2) A little more acid was formed and the pH was lower during the first stage of the mash with a pure culture than in the control mash; but after the middle stage, the pH rose, and buffer substances as well as relishing components were formed in a manner similar to that in the ordinary process.

3) Alcohol yield and the yield of "sake-cake" were not different from those in the ordinary process.

4) The quality of sake manufactured by the new process was not distinguishable statistically from that of an ordinary sample of sake.

3. Studies on the Brewing of Sake using Pure Cultures of Saccharomyces sake instead of "Moto". (III) Production of Yeast Cells by Tank

Culture (1): R. Miyazaki, O. Nagano, H. Yoshida, and M. Akaki. Jour. Soc. Brewing, Japan, 61 (6), 546-550 (1966).

In the previous papers we reported the manufacture of sake yeast, Saccharomyces sake, by shaking culture and the successful utilization of compressed cells of sake yeast as a substitute for "moto" in sake-brewing.

In this paper we introduced fermentation equipment devised by the authors for yeast culture and studied the production of sake yeast on an industrial scale using this fermentation equipment.

We put the principal object in manufacturing fermentation equipment on the following points, i.e., fitness, in scale, for moderate sake-brewing factory, aeration-agitation effectiveness of the propagation tank, prevention of contamination, and convenience in operation, etc.

The full capacity of the propagation tank was 240 liters and normal operating volume was about 100 liters.

With this fermentation equipment, oxygen absorption coefficient Kd (mol/ml. min. atm) was determined by the conventional sulfite method under various aeration-agitation conditions (aeration rate 50-200 l/min, agitation speed 100-300 r.p.m.). As a result, it was found that the agitation speed affected more greatly the Kd values of the propagation tank than did the aeration rate. The following empirial formula was made in the range of agitation speed of 200-300 r.p.m.:

$$Kd=11.4X10^{-12}V^{0.5}N^{1.7}$$

where: Kd is the oxygen absorption coefficient (mol/ml. min. atm); V is the air flow rate (l/min); N is the revolution number of agitation (r.p.m.).

Then, using this fermentation equipment, sake yeast Kyokai No. 7 was cultivated in media containing molasses, urea and salts under various conditions of aeration and agitation. Of the cultures studied, the better yields of the yeast were obtained from the cultures under the following conditions, i.e., aeration rate 50 l/min, agitation speed 300 r.p.m.; aeration rate 100 l/min, agitation speed 250 r.p.m.

By culturing the yeast in about a hundred liters of the molasses media containing 7.8% of total sugar under the aforementioned conditions, 8.5-9 kg of compressed yeast containing 76% of water, high in purity and light in color, was obtained.

XI Macdonald College, McGill University, Quebec, Canada. Communicated by Sister Cecily Mills.

The following is an abstract of a M. Sc. Thesis in Microbiology, which was recently submitted.

Pellicle Formation and Ester Production by Hansenula anomala
Cecily Mills

ABSTRACT

Three strains of Hansenula anomala, with different morphological and colony characteristics were studied with respect to pellicle formation and ester production. Approximately the same amount of top cells were produced by each strain. The top cells exhibited a greater respiratory activity than the bottom cells at the beginning of the fermentation. The use of inhibitors of pellicle formation showed that still cultures possessing no pellicle formed no ester but that ester was produced in shaken cultures in the presence of the inhibitor.

With increased aeration obtained by varying the surface-volume ratio, the peak of ester production occurred progressively earlier and the maximum ester values attained in the various flasks did not vary much with the different degrees of aeration. In the 'train' fermentations assuring almost complete ester recovery, one yeast strain produced equal amounts of ester in shaken and in still flasks as well as in 'foil-covered' flasks whereas another strain gave lower ester values in the shaken flasks probably because of the stronger esterase activity. The results obtained indicated that as much, if not more, ester is produced with increasing aeration.

XII Laboratory of Microbiology, Gulbenkian Institute of Science, Oeiras, Portugal. Communicated by Dr. N. van Uden.

1) This group, formerly at the "Departamento de Microbiologia, Instituto Botanico, University of Lisbon", moved into new facilities provided by the Gulbenkian Foundation. The Laboratory has departmental autonomy and covers a useful area of about 400 square meters in a new building with a total useful area of about 6.600 m^2. There are three other laboratories (Cellular biology, Physiology, Pharmacology), a large animal house, administrative services and about 1.500 m^2 laboratory space available for expansion. The institute is located in Oeiras, halfway between Lisbon and Estoril; it faces the 18th century palace of the Marques of Pombal (owned by the Gulbenkian Foundation) from which it is separated by a park kept in the original style.

The staff of the Laboratory of Microbiology is at this moment composed as follows: N. van Uden (Director); Lidia do Carmo Sousa (1st assistant); R. Castelo Branco (2nd assistant); Manuela Vidal Leiria (3rd assistant); J. França Mota (3rd assistant). In addition there are 7 technicians and temporary workers. Miss Vidal Leiria is in her second year at the University of California at Davis where she is working toward a Master's Degree in Microbiology under Dr. Phaff. Dr. Beryl Brady, formerly at the Brewery Research Foundation, Nutfield, England is with us on a Gulbenkian Fellowship.

2) Publications in the press

N. van Uden & J. Fell, Marine Yeasts in Droop & Ferguson Wood (Eds), Advances in Marine Microbiology, Academic Press, N. Y.

L. do Carmo Sousa, Endospore Formation in the Genera Trichosporon and Oosporidium. II International Symposium on Yeasts, Bratislava.

L. do Carmo Sousa & N. van Uden, Reisolation of Sarcinomyces inkin and its transfer to the genus Trichosporon, Mycologia.

H. R. Buckley & N. van Uden, Candida shehatae sp. n., a yeast associated with wood-destroying insects, Mycopathologia et Mycologia Applicata.

B. L. Brady, Effects of growth conditions on development of amidase activity in Candida utilis. II International Symposium on Yeasts, Bratislava.

M. Vidal Leiria, Candida mogii sp. n. a halotolerant yeast associated with Miso fermentation, Antonie van Leeuwenhoek.

N. van Uden, Transport-limited growth in the chemostat and its competitive inhibition; a theoretical treatment, Archiv für Mikrobiologie.

N. van Uden, Transport-limited fermentation and Growth of Saccharomyces cerevisiae and its competitive inhibition, Archiv für Mikrobiologie.

3) N. van Uden accepted to write a chapter on yeast ecology for a three volume treatise on the yeasts that is being prepared under the editorship of two well-known British microbiologists. All yeast workers who have published papers relevant to ecology are requested to send reprints.

XIII Southern Illinois University, Carbondale, Illinois. Communicated by Dr. C. C. Lindegren.

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

1) Lindegren, C. C. Circulation in the cell. Discovery 27: No. 8, 31-34 (1966).

2) Hwang, Y. L., Lindegren, G., and Lindegren, C. C. Genetic study of lysine biosynthesis in yeast. Canadian Journal of Genetics and Cytology 8: 471-480 (1966).

3) Hwang, Y. L. and Lindegren, G. Sites of affinity and linear arrangement of genes on chromosome V of Saccharomyces. Canadian Journal of Genetics and Cytology 8: 677-694 (1966).

4) Lindegren, Carl C. Cold War in Biology. Planarian Press, Inc., Ann Arbor, Michigan, pp 113 (1966).

5) Yau, Tommy and Lindegren, C. C. The melezitose locus in Saccharomyces: One gene producing more than one enzyme. Biochemical and Biophysical Research Communications 27, No. 3, 305-308 (1967).

6) Abstract: Satyanarayana, T., Umbarger, H. E. and Lindegren, G. Regulation of the leucine biosynthetic enzymes in yeast. Bacteriological Proceedings (1967).

Dr. Isamu Kondo has spent a little more than a year in the laboratory working on the host range specificity of the zymophage and has been successful in preparing supernatants which will completely destroy certain strains of yeast without affecting others. The electron microscopy on this subject has also progressed very successfully. Many of the supernatants that are particularly active were obtained from Rhodotorula. The electron microscope shows a clearcut difference in cell wall structure between Saccharomyces and Rhodotorula, but other characteristics are quite similar.

Dr. Isamu Ohkuro from the Jikei University School of Medicine, Tokyo, Japan, is continuing the work after a short period of instruction from Dr. Kondo, who has just recently returned to Japan. Generally speaking, the interest in this activity among the Japanese workers is quite considerable and very gratifying.

Dr. Lindegren is working on a book dealing with yeast cytology which will probably be completed in the course of the next several years.

Tommy Yau, a research assistant in the Biological Research Laboratory, has published a paper showing that the melezitose locus produces at least three separate enzymes, and there are recent indications that this same single gene locus produces at least one more enzyme.

The work on sites of affinity is progressing very favorably, and more data have been accumulated which will be analyzed this summer by Dr. Shult.

XIV University of Washington, Department of Microbiology, Seattle, Washington, 98105. Communicated by Dr. Howard C. Douglas.

When extracts of S. cerevisiae and S. fragilis are subjected to starch electrophoresis at pH 6.3 and stained to detect phosphoglucomutase activity entirely different patterns are revealed. Strains of S. cerevisiae possess two or three components which migrate toward the cathode, while strains of S. fragilis contain five to six components which migrate toward the anode. The S. cerevisiae pattern is displayed by several other species (S. carlsbergensis, S. italicus, S. chevalieri, S. diastaticus) with which it is interfertile, while the S. fragilis pattern is shared by S. lactis and S. marxianus. The latter three species are known from Wickerham's work to be interfertile and are thought by taxonomists to be only distantly related to S. cerevisiae.

A working hypothesis to explain these results is as follows. Each electrophoretic variant of phosphoglucomutase is controlled by a separate gene, the genes differing sufficiently from one another to specify proteins with a slightly different charge. These multiple loci may have arisen as a result of duplication of an original gene, the duplicated genes being subject to mutational alteration during evolution. If this explanation is correct, it would appear that S. cerevisiae and S. fragilis are, in fact, phylogenetically quite distinct since they have no genes in common which specify the synthesis of phospho-glucomutase.

XV University of Washington, Dept. of Microbiology, Seattle, Washington, 98105. Communicated by Mr. James N. Bicknell.

The following is a brief description of our current investigations on the DNA homology among species of Saccharomyces.

We are comparing the DNA homology among species of Saccharomyces sensu Lodder to determine their genetic relatedness. The DNA from 15 species has been compared to DNA from three reference species, S. lactis, S. fragilis and S. cerevisiae.

The results of the comparisons indicate that S. cerevisiae is only slightly related to S. fragilis since there is very little homology between their DNA molecules. The same is true for S. cerevisiae and S. lactis. S. lactis and S. fragilis DNA molecules are partially homologous. On the other hand, species interfertile with S. cerevisiae show complete or almost complete DNA homology with S. cerevisiae. Also, S. marxianus DNA is homologous with S. fragilis DNA. Many of the other species examined show no or only slight DNA homology with the DNA from the reference species.

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

"El-Tobshy, Zeineb M., and J. B. Sinclair, 1967. Effect of age of yolk-sac-inoculated embryonated chicken eggs on susceptibility to two isolates of Geotrichum candidum. Phytopathology 57: (in press) Abstr.

Yolk sacs of embryonated chicken eggs were aseptically injected with arthrospore suspensions in normal saline of 2 isolates of G. candidum using the hypodermic-needle-puncture technique. Isolates ATCC-7019 (citrus, American Type Culture Collection) and CH (human being) were injected into the yolk sacs of 6-, 8-, 10-, and 12-day-old embryos incubated at 31°C. Each treatment was replicated 10 times. Yolk sacs were found to be susceptible to both isolates with CH being the more pathogenic. Susceptability of embryos decreased with age. LD50 values (log-probability curves) were determined 3 days after inoculation. For ATCC-7019, LD50 values for 6-, 8-, 10-, and 12-day-old embryos were: 20,000, 80,000, 150,000 and 700,000, arthrospores/ml. respectively. For CH these values were: 5,000, 13,000, 17,000 and 47,000 arthrospores/ml., respectively. This was the first report of a phytopathogen being cultured on yolk sac of embryonated chicken eggs. The technique proved to be a means for measuring virulence between isolates of the fungus.

XVII Dept. of Food Science and Technology, University of California, Davis, Calif. 95616. Communicated by Dr. H. J. Phaff.

Dr. Ahmed T. H. Abd-el Al obtained the Ph. D. degree in the field of Microbiology, May 1967. The research was done under the guidance of Prof. H. J. Phaff. A summary of the thesis "Purification and properties of beta-glucanases of yeast" follows below.

1) A number of yeast species were examined for the presence of β-glucanases.

Extracts obtained by cell disruption of Saccharomyces cerevisiae, S. elegans, S. (Fabospora) fragilis, and Hansenula anomala hydrolysed laminarin and pustulan with the production of glucose (exo-β-glucanase or exo-BG), whereas extracts of Hanseniaspora valbyensis and Hanseniaspora uvarum only hydrolysed laminarin with the production of oligosaccharides (endo-β-glucanase or endo-BG). Enzymic activities were also detected in culture fluids of S. fragilis, H. anomala, and H. valbyensis, but the culture fluid of S. cerevisiae contained negligible amounts.

2) S. fragilis, H. anomala, and H. valbyensis are yeasts whose asci lyse rapidly upon maturity, thus liberating the ascospores, whereas the ascus walls in S. cervisiae and H. uvarum remain intact until they are ruptured by what has been interpreted as a swelling process of the spores during germination. These two groups of yeasts differ greatly in their β-glucanase activity. For example, S. fragilis possessed approximately 7-times higher β-1→3 glucanase activity (per g dry wt. of cells or mg protein in the extract) than S. cerevisiae.

3) The extracellular enzyme level of a strain of S. fragilis was maximal after 24 hrs of aerobic growth at 30° in a complete mineral medium with 5% glucose and 0.05 M Na-succinate buffer at pH 6.0. A variety of carbon sources (including laminarin and cell walls) failed to induce a higher level of the enzyme in the culture fluid. Two- to three-fold increase in specific activity of exo-BG was obtained with glycerol as carbon source, but the slow growth with this carbon source made its use impracticable.

4) Exo-BG from X. fragilis was purified 114-fold from the dialysed culture fluid by column chromatography on DEAE-cellulose, followed by Sephadex (G-100) gel filtration. The enzyme was also purified from the dialysed intracellular extract. This was done by heating, protamine sulfate precipitation, DEAE-cellulose and Sephadex G-75 chromatography and resulted in 423-fold purification. The purified extracellular and intracellular enzymes had essentially the same specific activity.

5) Intracellular exo-BG from baker's yeast was purified 344-fold from the dialysed extract by heating, chromatography on DEAE-cellulose, and on Sephadex G-100.

6) Extracellular exo-BG of H. anomala was purified 600-fold by chromatography on DEAE-cellulose and Sephadex G-75.

7) The optimal pH of the enzymes from S. fragilis, S. cerevisiae, and H. anomala was 5.5 in each case. Chromatographic evidence indicated that the three enzymes sequentially remove glycosyl units from laminarin as well as pustulan. They can be classified therefore as exo- or endwise-splitting enzymes.

8) The ratio of activities toward laminarin and pustulan remained constant during purification of the exo-BG obtained from the three species. This suggested that a single enzyme possesses both β-1→3- and β-1→6-glucanase activities. Additional evidence for its uni-enzymatic nature has been obtained. (1) The two activities were destroyed at exactly the same rate upon heating of the purified enzyme from S. fragilis at three

different temperatures. (2) The competitive inhibitor glucono-δ-lactone gave the same value of K_i when tested with either substrate. (3) Quantitative application of the "mixed substrate" method with the purified enzyme of S. cerevisiae gave evidence that both activities reside in a single enzyme.

9) The purified exo-β-glucanases of the different species of yeast have different kinetic constants. Ratios of maximal velocities and K_m values with laminarin and pustulan differed markedly. Comparison of V_{max} and K_m values suggested that the rapid release of spores from asci in S. fragilis might be explained in terms of an enzyme with higher maximal velocity and higher affinity to the ascus wall than the exo-BG present in baker's yeast.

10) The estimated molecular weights for exo-BG from S. fragilis, S. cerevisiae, and H. anomala were 22,000, 40,000, and 30,000, respectively, based on results obtained with gel filtration.

11) The properties of extracellular and intracellular exo-BG were similar in S. fragilis as well as in H. anomala.

12) The purified exo-BG from S. fragilis hydrolysed 0.2% laminaribiose at approximately 1/3 of the calculated maximal velocity of the enzyme with laminarin. Gentiobiose, however, is hydrolysed at a rate far lower than is pustulan by the enzymes of S. fragilis, S. cerevisiae, and H. anomala.

13) β-glucanases from H. valbyensis and H. uvarum were found to be randomly splitting enzymes. They hydrolysed laminarin but not pustulan or other β-glucans.

14) The extracellular enzyme level of H. valbyensis was maximal after 48 hrs of aerobic growth at 30° in a complete mineral medium with 3% glucose and 0.2 M Na-succinate buffer at pH 5.5. The amount of the enzyme was dependent on the level of aeration and the molarity of the buffer. The use of 1% laminarin or 1% baker's yeast cell walls plus 0.1% glucose in 0.2 M buffer failed to induce higher levels of the enzyme.

15) Extracellular endo-BG from H. valbyensis was purified 34-fold by column chromatography on DEAE-cellulose.

16) The endo-BG of H. valbyensis had a broad pH optimum between pH 3.5 and 4.5.

17) Endo-BG of H. valbyensis did not hydrolyse oat glucan, which suggested that the enzyme may require two or more consecutive β-1→3 bonds for action. No action was detected with laminaribiose or laminaritriose. Laminaritetraose, however, was split into laminaritriose + glucose or into two moles of laminaribiose.

XVIII Brief News Items

1. "As a result of the impending closure of the Low Temperature Research Station, in Cambridge, England, J. A. Barnett and M. Ingram

are separating. J. A. Barnett was recently awarded a Ph. D. degree in Cambridge, for a dissertation entitled, "Critical studies in yeast taxonomy"; and he has been appointed to the staff of the Agricultural Research Council's new Food Research Institute to be built at Norwich. He recently went on a working visit to the Czechoslovak Academy of Sciences, Prague. M. Ingram is going to Langford, Somerset, England, to become Director of the Agricultural Research Council's new Meat Research Institute there."

M. Ingram
Low Temperature Research Station
Downing Street
Cambridge, England

2. Since my retirement in June 1966 from the National Institutes of Health, I have been busy with committee work, lecturing and traveling. On June 14 Mrs. Emmons and I go for 4 or 5 months to work as volunteers in Hospital Amazonico "Albert Schweitzer", Pucallpa, Peru, where I shall be looking for tropical mycoses and doing some teaching.

C. W. Emmons

3. I take pleasure in announcing that a modern research laboratory building has been erected at Takasaki, Gumma Prefecture, Japan and the opening ceremony will be held on April 5, 1967. Both the Laboratories located in Yokohama and Amagasaki have been integrated into this building and they have been closed.

The business of The Research Laboratories of Kirin Brewery Co., Ltd. is to be carried on thereafter at the above residence. Please address all communications to the above in the future. I am looking forward to your visit to us at any time.

With my best regards,

Yours very sincerely,

Dr. Yoshiro Kuroiwa
Director of Research

4. Production of Candida utilis by Charmin Paper Products Company at Green Bay, Wisconsin, was discontinued in May 1967.

MICROBIAL TECHNOLOGY is a new book updating industrial microbial practices. It is the collaborative effort of 22 specialists - microbiologists, biochemists, bioengineers - who have written authoritatively about their own areas of applied microbiology. This book, edited by H. J. Peppler, will be published in August 1967 by Reinhold Publishing Company, New York.

Henry J. Peppler
Universal Foods Corporation
Milwaukee, Wisconsin

5. The following article was published in Food Manufacture, Sept. 1966, 3 pp.

"Yeast Products, and their role in food development". Subheadings: Sources of yeast, Yeast constituents, Production of Yeast Extract (autolysis, plasmolysis, hydrolysis, further processing), Applications (flavor enhancing).

E. East, B. J. Smith, and D. G. Borsden
English Grains Ltd.
Granary House, Burton-on-Trent
England

6. The Proceedings of the International Symposium on Yeast Protoplasts held in Jena, D.D.R., Sept. 21-24, 1965, were published April 1967. The mailing of reprints began in May. The proceedings appear in the "Abhandlugen der Deutschen Akademie der Wissenschaften zu Berlin, Klasse für Medizin, Jahrgang 1966, No. 6. Printed by Akademie Verlag, Berlin (East).

Rudolf Müller
Jena, D.D.R.

7. The following papers have been published recently:

Étude de la croissance anaérobie des levures au cours de vinifications par macération de raisins entiers. J. Chauvet, P. Bréchot, M. Croson et R. Irrmann. Ann. Technol. agric. 1966, 15 (1), 9-111.

Extrait de pruine de raisin, facteur de croissance anaérobie de la levure, cultivée sur moût de raisin. P. Bréchot, J. Chauvet, M. Croson, and R. Irrmann. C. R. Acad. Sc. Paris 263, 1004-1006 (Serie D)

Paul Bréchot
Institut Pasteur
25, Rue du Docteur Roux-
Paris XV, France

8. The following publications have appeared:

M. BESSON
Les membranes des ascospores de levures au microscope électronique. Bull. Soc. Mycol. France 82, 489-503, 1966

C. PONCET
Etude taxométrique du genre Pichia Hansen (Ascomycètes, Saccharomycetaceae) C. R. Acad. Sc. Paris série D 264, 43-46 1967

J. B. FIOL
Les besoins vitaminiques dans les genres Debaryomyces Kloecker, Schwanniomyces Kloecker, Hansenula H. et P. Sydow et Endomycopsis Dekker. C. R. Acad. Sc. Paris série D 264, 1605-7, 1967

M. C. PIGNAL
Une nouvelle espèce de levure isolée de larves d'insectes Pichia stipitis. Bull. mens. Soc. Linn. Lyon 36 (4), 163-168, 1967

M. BESSON
La fructification d'Ascocybe grovesii Wells (= Cephaloascus fragrans Hanawa) au microscope électronique. Bull. mens. Soc. Linn. Lyon 36 (6), 230-3, 1967

In the next issue of "Antonie van Leeuwenhoek" the following article will appear:

S. PONGET
A numerical classification of yeasts of the genus Pichia Hansen by a factor analysis method (14p)

Dr. J. Boidin
Université de Lyon
Lab. de Biologie Végétale
69 Villeurbanne, France

9. Dr. H. Suomalainen reports the following publications on yeast from the Research Laboratories of the State Alcohol Monopoly, Helsinki, Finland.

The aroma components produced by yeast in nitrogen-free sugar solution. H. Suomalainen and L. Nykänen. Jour. Inst. Brewing 72: 469, 1966.

The aroma compounds produced by sherry yeast in grape and berry wines. H. Suomalainen and L. Nykänen. Suomen Kemistilehti B39: 252, 1966.

Dicarbonyl compounds of whisky and cognac. P. Ronkainen and H. Suomalainen. Suomen Kemistilehti B39: 280, 1966.

10. Drs. H. J. Phaff and M. W. Miller, Dept. of Food Science and Technology, University of California, Davis, are the American participants of a joint U.S.-Japan cooperative Science Project, sponsored by the U.S. National Science Foundation and the Japan Society for the Promotion of Science. The Japanese scientists collaborating in the project are Dr. M. Yoneyama, Hiroshima University; Mr. M. Soneda, Nagao Institute, Tokyo; Dr. K. Kodama, Kodama Brewing Laboratories, Iidagawa (Akita Pref).

The title of the project is "Systematic and Ecological Investigations on Fungi in the Pacific Region, especially the groups common to Japan and the U.S.A. II. The yeasts occurring in exudates of trees and related habitats."

This spring Dr. Miller spent 9 weeks in Japan and Dr. Phaff 5 weeks. Yeasts were collected from the extreme southern part to the very north. Half of the approximately 1500 cultures obtained will be identified in Japan and the other half in Davis.

In August Drs. Yoneyama and Soneda will come to Davis for several weeks to discuss identification procedures and other aspects of the project.

11. North Holland Publishing Co., Post Office Box 103, Amsterdam, Netherlands.

Lodder, J. and Kreger-van Rij, N.J.W., Delft, The Netherlands.

The Yeasts. A taxonomic study, first printing 1952, second printing 1967. 725 pages, 268 figures, cloth Hfl. 100.--; $28.00.

Contents: Introduction. Characteristics and methods used in the classification. Variation in yeasts and its significance in taxonomy. General classification of the yeast. Discussion of the genera belonging to the Endomycetaceae. Discussion of the genera Sporobolomyces and Bullera. Discussion of the genera belonging to the Cryptococcaceae, Latin diagnoses of new genera, species and varieties. Author index. Index to the names of taxa.

12. Miscellaneous publications.

Symposium: Microorganisms as potential food sources. Developments in Industrial Microbiology, Vol. 7, 1966. (Introduction by William D. Gray, Chairman, followed by discussions of six panelists.)

Oligonucleotid-Aufbau als biochemisch faszbares Indiz für den Beginn der Knospung bei Saccharomyces cerevisiae. H. Kleinkauf. Archiv f. Mikrobiologie 56: 167-192, 1967. [Botanisches Institut der technischen Hochschule, Braunschweig, W. Germany].