The four sporulation media gave heavy yields of asci, with 1, 2, 3, and 4 ascospores per ascus, after a few days of incubation at 25 C. Moreover, five subcultures, VT1–2 to VT1–6, isolated from a fermentation of VT1 in yeast water with 29 per cent glucose.

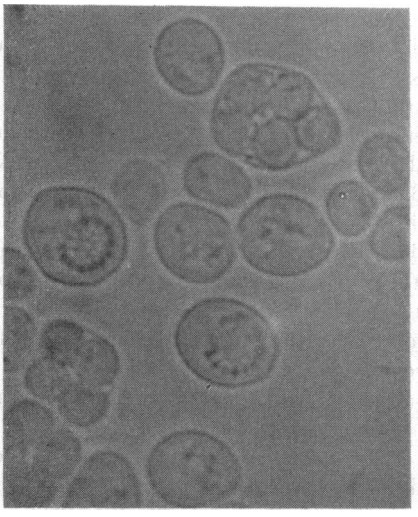

Figure 2. Saccharomyces cerevisiae strain VT1. Asci with 4 and 5 ascospores. From Kleyn medium at 3 days at 25 C. Unstained. × 2000.

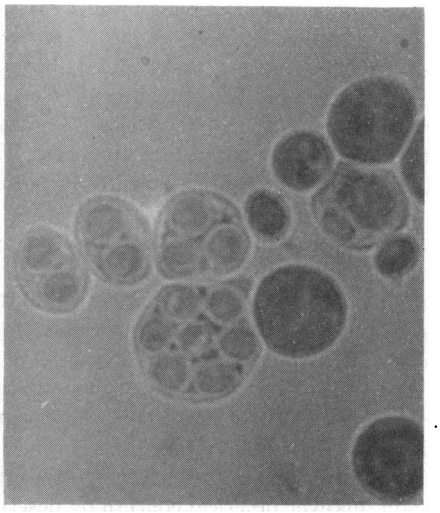

Figure 3. Saccharomyces cerevisiae strain VT1-6. Asci with 2, 3, and 7 ascospores. From Kleyn medium at 5 days at 25 C. Malachite green and safranin stain after fixation in fluid of Luria. × 2000.

on Kleyn medium, asci with 5, 6, 7, and 8 round, smooth ascospores (figures 1–3), are formed. Likewise round or short-oval cells longer than usual (7–9 μ by 8.5–10 μ), are formed on this medium.

The same formation of asci with 5 to 8 ascospores in Kleyn medium was found with I have found the same asci with more than 4 spores on Kleyn medium, in another strain of *S. cerevisiae*, strain VB12-2, isolated from a white wine.

CATALASE ACTIVITY IN *PEDIOCOCCUS CEREVISIAE* AS RELATED TO HYDROGEN ION ACTIVITY

R. R. GUTEKUNST,[1] E. A. DELWICHE, AND H. W. SEELEY

Laboratory of Bacteriology, College of Agriculture, Cornell University, Ithaca, New York

Received for publication May 13, 1957

Catalase, present in most microorganisms but presumably absent in a few important groups, is routinely determined qualitatively in many laboratories. Its absence has been considered to be significant in establishing the taxonomic position of unknown cultures, particularly among the members of *Lactobacteriaceae*. Recent studies with the pediococci (Felton *et al.*, J. Bacteriol., **65,** 481, 1953; Jensen and Seeley, J. Bacteriol., **67,** 484, 1954) have revealed that the group differs from other lactic acid bacteria, among other characteristics, in that this enzyme, hitherto overlooked, is indeed demonstrable in most cultures of pediococci. Felton *et al.* (J. Bacteriol., **65,** 481, 1953) regard this positive catalase

[1] Lieutenent, U. S. Navy. The opinions expressed in this report are the private ones of the authors and are not to be construed as reflecting the views of the Navy Department or the Naval Service at large (Article 1252, U. S. Navy Regulations, 1948).

reaction as offering some cause for retention of the group within the family *Micrococcaceae*; but, in spite of this work, Pederson (Bacterol. Revs., **13,** 227, 1949), in the opinion of the authors, marshals ample justification for the reclassification of the genus *Pediococcus* into *Lactobacteriaceae*. Recent reports (Prévot and Raynaud, Ann. Inst. Pasteur, **88,** 229, 1955; Dacre and Sharpe, Nature, **178,** 700, 1956) of catalase positive lactobacilli have also appeared. These findings of catalase positive cultures of both the genera *Pediococcus* and *Lactobacillus* cast real doubt on the validity of the catalase test as an overriding classification feature, and by an extension of this view reemphasize the limitations of any single physiological characteristic in the attempts to establish natural categories, a concept so adequately developed by Sherman (Bacteriol. Revs., **1,** 1, 1937) in his monograph dealing with the streptococci.

It is the purpose of this brief report to point up again this ever practical concern by describing certain conditions which profoundly affect the catalase test in pediococci. We shall establish that the common practice of simply growing the organism on an agar plate, flooding the colonies with dilute hydrogen peroxide, and watching for the evolution or nonevolution of oxygen may be completely inadequate, both as a test for the enzyme, and in establishing the latent ability of the organism to produce the enzyme.

Four strains of pediococci were studied, including strain 8081 which was reclassified from the genus *Leuconostoc* into the genus *Pediococcus* by both Felton and Niven (J. Bacteriol., **65,** 482, 1953) and Jensen and Seeley (J. Bacteriol., **67,** 484, 1954) after having been found to be catalase positive under certain specific growth conditions, as well as having physiological and nutritional characteristics typical of the genus *Pediococcus*. All strains were classified as *Pediococcus cerevisiae*. When grown on the medium described by Evans and Niven (J. Bacteriol., **62,** 599, 1951), hereafter designated as the high glucose medium, all cultures were catalase negative on agar plate culture as tested by the usual procedure of flooding the plates with 3 per cent hydrogen peroxide. When grown on a solid culture medium containing a lower concentration of glucose, however, (0.05 per cent versus 1.0 per cent) poor growth resulted, but catalase activity could be detected in 3 of the 4 strains by the conventional procedure. Strain F166 was negative. This medium of Felton *et al.* (J. Bacteriol., **65,** 481, 1953), hereafter referred to as the low glucose medium, was found to be much more favorable to the development of catalase positive cultures. In composition it contained tryptone, 1%; yeast extract, 0.5%; NaCl, 0.5%; K_2HPO_4, 0.2%; and glucose, 0.05% (pH 6.8). Subsequent experimentation established that the principal factor involved in depressing catalase activity was acid production, which was much more pronounced in the high glucose medium. This medium contained a 20-fold greater concentration of glucose, was

TABLE 1

Catalase activity before and after readjustment of pH of cells grown at 30 C and 37 C in low glucose and high glucose broth

Strain	Temperature	High Glucose Medium				Low Glucose Medium			
		Before pH adjustment		After pH adjustment		Before pH adjustment		After pH adjustment	
		pH	Catalase activity*	pH	Cataase activity*	pH	Catalase activity*	pH	Catalase activity*
	C		*cm*		*cm*		*cm*		*cm*
F-166	37	4.6	0	7.0	10	6.8	0	4.5	0
8081		4.5	0	7.0	4	6.8	4	4.5	0.5
N-82		4.5	0	7.0	10	6.6	10	4.5	4
E-66		4.6	0	7.0	4	6.8	4	4.5	0
F-166	30	4.6	0	6.9	2	6.8	0	4.4	0
8081		4.5	0	6.9	10	6.7	4	4.4	0
N-82		4.7	0	6.9	10	6.7	4	4.4	4
E-66		4.8	0	6.9	10	6.7	2	4.5	0

* Approximate length of oxygen column after 60-min incubation.

more favorable for growth because of its extensive supplementation with growth factors, but almost invariably yielded catalase negative cells both on solid and liquid media. In liquid culture, the low glucose medium gave catalase positive cultures with 3 of the 4 strains. Measurement of catalase activity under these growth conditions was made by introducing 1 ml of 3 per cent hydrogen peroxide into 15 ml of 24 hr culture (30 C) in a Smith fermentation tube, and observing for oxygen evolution in the side arm. Strain F166 was consistently catalase negative; strain E66 was weakly positive; and strains 8081 and N82 were strongly positive. In contrast, on the high glucose medium only 1 culture (N82) showed a very weakly positive reaction. With this medium the final pH of growth was in the range of 4.7 to 5.0, whereas with the low glucose medium the pH was always in the range of 6.7 to 6.8; thus, a hydrogen ion effect is strongly implicated.

Further evidence substantiating an effect of hydrogen ion concentration in controlling the enzyme development as well as its manifestation is presented in table 1. Cultures were grown on both media, catalase activity was measured, the pH was adjusted in duplicate cultures to near neutrality in the case of the cultures on the high glucose medium and to pH 4.4 to 4.5 in the case of the cultures grown on the low glucose medium, and then catalase activity was measured in these cultures in which pH adjustment had been made. It was observed that pH adjustment upward toward neutrality enhanced activity and pH adjustment downward diminished activity. The results in table 1 generally demonstrate that the pH effect is primarily exerted on the activity of the enzyme system rather than on enzyme development.

It might be argued, however, that a minor causative effect on enzyme development (or destruction) may be exerted, since the downward pH adjustment of the cultures grown on the low glucose medium yielded slightly active cells whereas those cultures grown on high glucose media (unadjusted) were negative.

Cell permeability does not appear to be involved, since in no case did acetone treatment appear to affect the catalase activity greatly.

INFRARED ABSORPTION STUDIES OF PURIFIED NORMAL AND VIRUS INFECTED MOUSEBRAIN SUSPENSIONS[1]

B. V. SIEGEL, Y. C. NG,[2] N. K. FREEMAN, AND W. L. BOSTICK

Department of Pathology, University of California School of Medicine, San Francisco, and Donner Laboratory of Biophysics and Medical Physics, Berkeley, California

Received for publication May 27, 1957

The present status of infrared absorption spectroscopy of viruses was recently reviewed by Benedict (Ann. N. Y. Acad. Sci., *in press*). He concluded that the method possessed potential usefulness in the characterization of virus preparations of high purity.

In the present studies the virus employed represented an 18th mousebrain passage of an agent isolated after 6 to 8 intracerebral passages of Hodgkin's disease granuloma filled lymph nodes in newborn Princeton strain albino mice (Bostick and Hanna, Cancer Research, **15,** 650, 1955). Parenthetically, a direct relationship between this virus and Hodgkin's disease has yet to be definitely established. Electron micrographs of virus preparations purified by butanol extraction and a single cycle of differential centrifugation (Bachrach and Schwerdt, J. Immunol., **69,** 551, 1952) revealed two different sized, approximately spherical shaped particles, one 31 mμ in diameter and the other 17 mμ; while those of purified normal mousebrain preparations contained only the 17 mμ particle (Bostick and Siegel, Am. J. Clin. Pathol., *in press*).

The methodology of infrared spectroscopy was undertaken in an attempt to distinguish biochemically these two particles. Additionally,

[1] This investigation was supported in part by the U. S. Atomic Energy Commission and by a grant from the Rosenstiel Foundation.

[2] Public Health Service Research Fellow of the National Heart Institute.