MARINE-OCCURRING YEASTS OF THE AVALON PENINSULA, NEWFOUNDLAND: THEIR IDENTIFICATION AND VERTICAL AND SEASONAL DISTRIBUTION

BARBARA ANN SWYERS

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by

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A Thesis submitted in partial fulfilment
of the requirements of the degree of
Master of Science

Department of Biology
Memorial University of Newfoundland
November 1975

St. John's Newfoundland

ACKNOWLEDGEMENTS

I wish to acknowledge the financial support given me in the form of a Memorial University fellowship. I would like to thank my supervisor, Dr. George Moskovits for his guidance and encouragement. Ms. Anne Moore carried out all of the fieldwork from November 1972 to June 1973; and that part of the laboratory work up to and including the plating of the plankton samples before she left the project in the autumn of 1973. Mr. Wayne Burke of the Marine Sciences Research Laboratory and Ms. Joyce Noseworthy were of great assistance not only with their knowledge but also with transportation to and from the laboratory. I would like to thank Dr. Sean Brosnan and Mr. Doug Hall of the biochemistry department at Memorial University for their help with the biochemical techniques involved in this study. I wish to thank Ms. Judy Conway for the typing of the manuscript and Dr. Derek Jones for proofreading and constructive criticism.

ABSTRACT

Yeasts were isolated from plankton samples collected off Small Point, Avalon Peninsula, Newfoundland. Morphological, reproductive, cultural and biochemical characteristics were examined. Identification was carried out by means of a key developed by Barnett and Pankhurst (1974) using biochemical data as diagnostic criteria. Only after all data were examined was identification considered final but the use of the biochemical key avoided the more subjective evaluations necessary when depending upon morphological, cultural and reproductive characteristics.

The following yeats were identified: *Debaryomyces hansenii*, *Rhodotorula rubra*, *Candida diddensii*, *Rhodotorula glutinis* var. *glutinis*, *Candida diffluens*, *Candida tropicalis*, *Rhodotorula graminis*, *Cryptococcus albidus* var. *albidus*, *Cryptococcus infirmo-miniatus*, *Candida humicola*, *Candida maritima* and *Leucosporidium scottii*. Several "black yeasts" were also isolated. None of the species identified were unique to Newfoundland or northern marine waters. Unidentified isolates were either keyed out to be organisms differing in most of the other criteria or those which had too many questionable reactions to be definitely assigned to a genus and species.

Methodology, as well as possible effects of environmental factors on the growth of the isolates were discussed and the marine distribution of the species identified was reviewed.

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INTRODUCTION

Yeasts do not comprise a clearly defined group of organisms and a definition of the group is somewhat difficult. Yeasts are positioned within the phylum Eumycophyta and are the most primitive of the Ascomycetes, being characterized by the formation of naked asci. Sexual reproduction may occur but yeasts can also reproduce asexually by fission or budding.

There are 39 genera of yeasts and, at present, 442 species. In the present study, Lodder's (1970) taxonomy was used as the basis for classification. Lodder divided the yeasts into four groups, the ascomycetous yeasts with asci and ascospores, the Ustilaginales including *Rhodosporidium* and *Leucosporidium*, ballistosporogenous yeasts in the family Sporobolomycetaceae, and the asporogenous yeasts, as in the family Cryptococcaceae.

Ascospore formation has been of great importance when using Lodder's key. If ascospores are formed, identification can be relatively straightforward. However, if they are not produced it does not necessarily mean that a yeast is asporogenous. Such a yeast may have been grown on media unsuitable for sporulation, may have lost its ability to form ascospores, may form only a few

ascospores which may be difficult to find or may be a heterothallic haploid.

The key used in the present study was developed by Barnett and Pankhurst (1974) using a computer and is designed to help the individual with the problem of identifying hundreds of yeast isolates. It is based on the yeast cell's reactions to physiological tests and eliminates, to a certain extent, the dependence on morphological and cultural data which involve a more subjective evaluation than the per cent transmission reading obtained from a spectrophotometer or the presence or absence of growth on a slant or a plate. However, a cautionary note is given to examine all morphological, reproductive and cultural data before assigning a name to the yeast in question. The new key, which includes information on 93 additional species, avoids the dependence on ascospore formation prevalent in Lodder (1970) but it must be accompanied by this key.

Lodder's key depends firstly on morphological and reproductive characteristics and then on physiological characteristics. It is, thus, more subjective and time-consuming but Barnett and Pankhurst's key used in conjunction with Lodder's key provide an excellent guide for identification.

A number of schemes, other than the two just discussed, have been developed to classify yeasts but most have been limited in scope and of little practical value.

In 1954, Kudriavzev published a classification of the sporogenous yeasts, basing much of his classification on the shape of spores. This characteristic will undoubtedly be of continuing importance in yeast taxonomy especially with the use of the electron microscope which will give detailed information on the composition of the spore wall and its configuration. However, this approach is still somewhat impractical for large scale identification purposes.

As far as numerical taxonomy is concerned, Poncet (1967) described the numerical classification of the genus Pichia by a factor analysis method. However, assigning equal values to the results of a number of tests is a risky business since so little is known about the actual interdependence on various systems within yeast cells. One reaction could affect another giving a misleading result.

Another method used in classification has been studying the DNA base composition of the yeast cell. Storck (1966) determined the base composition (G+C) content of 30 species of fungi including 12 yeasts. He found that the GC content of fungal DNA ranged from 38 to 63 per cent but this range

decreased from classes to subclasses to genera and species. Also, Nakase and Komagata (1968) examined the DNA base composition of 140 species of yeasts, showing marked differences in (G+C) content between sporogenous and asporogenous yeasts. Cryptococcus and Rhodotorula exhibited high GC contents, 46 to 56 per cent and 47.5 to 65.5 per cent respectively. In the genera, Torulopsis, Candida and Trichosporon, GC contents covered a range from 28 to 60 per cent indicating the heterogeneity of these genera. This provides more valuable information not only about the classification of yeasts but also concerning their evolutionary relationships.

Novak and Zsolt (1961) published a new classification scheme for both sporogenous and asporogenous yeasts. This was based on what they believed to be the evolutionary development of yeasts. They used descriptions of species provided by other researchers and since there are differences in methodology and classification systems, it is not a unified or accurate classification scheme.

Tsuchiya, Fukazawa and Kawakita (1965) introduced a method of classifying yeasts by means of serology. They tested 140 species plus a number of strains of each species and carried out antigenic analyses, the results of which depend to a great extent on the constitution of the cell

wall. However, many yeasts which are closely related have been placed in different antigenic groups to leave us questioning all the data previously obtained on the species or the total validity of this serological technique.

Generally speaking, classification and identification require an evaluation of all available data on an ever-enlarging number of physiological, cultural, reproductive and morphological observations. In the present study, all available date were taken into consideration before identification of a yeast isolate was finalized.

The literature has been somewhat devoid of material concerning the isolation of yeast species in northern or cold marine waters. Although many researchers have reported the presence of yeasts in their samples, few have attempted identification.

One of the most important researchers in the field of yeast species distribution in marine waters has been A. E. Kriss. Kriss and Novozhilova (1954) collected plankton and water samples from the northwestern Pacific Ocean and the sea of Okhotsk. Yeasts were found in 50% of the plankton samples but in only 40% of the water samples from the Sea of Okhotsk and in only 14% of water samples collected in the northwestern Pacific area. All species isolated were asporogenous and were classified as species of Torulopsis,

Rhodotorula and Sporobolomyces.

In 1954-56, Kriss also carried out microbiological studies of the Arctic Ocean and found colonies of white and pink yeasts. Studies in the Greenland Sea included isolation and identification of Debaryomyces globosus, Cryptococcus aerius, Saccharomyces rosii and Pichia guilliermondii (Kriss, 1963). He also described horizontal distributions of various species isolated in his studies and suggested that the marine-occurring species may be forms that have become adapted to life in a marine environment.

Morris (1968) has also done considerable work not only on the isolation and identification of marine-occurring yeasts but also reviewing the data already collected to give a relatively comprehensive summary of the geographical distribution of various species.

Considerable work examining physiological aspects of yeasts identified from samples collected off the Swedish west coast, considered to be a north temperate zone has been done by B. Norkrans. Of the water samples studied in one survey (Norkrans, 1966a) only 60% contained yeasts. Debaryomyces hansenii and Rhodotorula and Cryptococcus species predominated. From the collections made here, a new species, Candida suecica was isolated. (Rodrigues de Miranda and Norkrans, 1968).

Meyers, Ahearn, Gunkel and Roth (1967) did an extensive investigation of the yeast populations of the North Sea from 1964 to 1966. The predominant species found was Debaryomyces hansenii. Other yeasts identified included Rhodotorula rubra and Candida diddensii. There appeared to be an increase in the concentration of yeast cells during the summer months when Noctiluca blooms occurred.

An extensive microbiological study of the northeastern and the southeastern Atlantic Ocean was done by Fischer and Brebeck (1894). They were among the first to report the occurrence of yeasts in sea water and they classified most of them as "Torula" species. They did however describe some "black yeasts" as well.

Tsiklinsky (1908) isolated two yeast species on his Antarctic voyage and Issatchenko (1914) reported a general occurrence of yeasts in Arctic waters. He described "black yeasts" in one study at depths of five to ten meters. Off the Murmansk coast in the Arctic Ocean, Nadson and Burgwitz (1931) isolated 22 species of yeasts classifying most of them as "torula" species.

Shinano (1962), working in the north Pacific area, isolated yeasts in 14.4% of his water samples. They were found at all depths and subsequent identification showed Rhodotorula rubra, Rhodotorula glutinis and Torulopsis dattila to be the predominant species.

Obligately psychrophilic species tentatively identified as Candida and Torulopsis were isolated from Antarctic sea water samples by Sinclair and Stokes (1965).

Goto, Sugiyama and Iizuka (1969) studied yeasts from various locations in the Antarctic. The oceans surrounding the continent were not examined but one of the lakes, Lake Vanda, showing a high salinity revealed the presence of Cryptococcus albidus, Trichosporum cutanum var antarticum, two species of Candida and two species of Rhodotorula.

Fell, Statzell, Hunter and Phaff (1969) examined yeasts obtained from Antarctic water samples and found two overlapping groups of species: a heterothallic species, Candida scottii and a proposed new genus, Leucosporidium with two heterothallic species and five self-sporulating species.

A mycological study of Long Island Sound and the Housatonic River Estuary was carried out by Combs, Murchelano and Jurgen (1971). In the Long Island Sound area, the predominant species of yeasts were found to be Candida. Other species belonged to the genus Rhodotorula.

Some researchers have isolated yeasts from marine sources other than water samples. Hunter (1920) reported a shipment of oysters from New England being spoiled by the presence of a "pink yeast". However, the latter was also found in healthy oysters. Such yeasts were also reported found on

frozen oysters by McCormack (1950) who stated that they could grow at temperatures below freezing. Upon examining North Pacific salmon, Snow and Beard (1939) found up to 1% of the organisms present to be yeasts. Dyer (1947) studied micrporganisms isolated from Atlantic cod collected in Nova Scotia waters and 6% of the isolates were found to be yeasts but they were not classified. Hagan and Rose (1961) isolated a Cryptococcus (which they described as psychrophilic) from Laminaria species gathered from a beach in a Labrador fjord.

Species of Debaryomyces hansenii, Torulopsis candida and Trichosporon cutanum were isolated from fish and invertebrate material collected in the eastern north Atlantic area by Siepmann and Hohnk (1962). The predominant species was Debaryomyces hansenii.

Ross and Morris (1965), working on fish in the Firth of Clyde, Scotland, isolated Torulopsis inconspicua (var.), Candida parapsilosis, Rhodotorula glutinis var. rubescens and several other species but the predominant species was Debaryomyces kloeckeri. The latter was also the predominant species found upon examination of fish from the North Sea. This was again the case in fish collected from the Atlantic Ocean off Iceland. Other species isolated from this area included Candida parapsilosis and Rhodotorula glutinis.

Psychrophilic yeasts were isolated from marine fish by Bruce and Morris (1973). Of the several species of yeasts isolated, 25% were psychrophilic growing at 4C but not at 30C.

The advent of a key based on biochemical data, rather than the tedious and subjective morphological data, may encourage more work in the area of identification of yeasts isolated from natural habitats.

Marine waters and their contents have become of vital interest in the past decade and it is essential to increase our knowledge of the seas by studying the microbiological life which constitutes such an important part of the seas' total biological productivity.

It is becoming more evident that environmental conditions should be included in the data collected to show any effects created by changes in these conditions. Morris (1975) proposes that future studies examine these effects more carefully.

No studies of yeast populations have previously been carried out in Newfoundland waters. In the present study, an attempt has been made to examine and identify various yeasts isolated from these waters.

MATERIALS AND METHODS

A. Field Work

1. Sampling Site

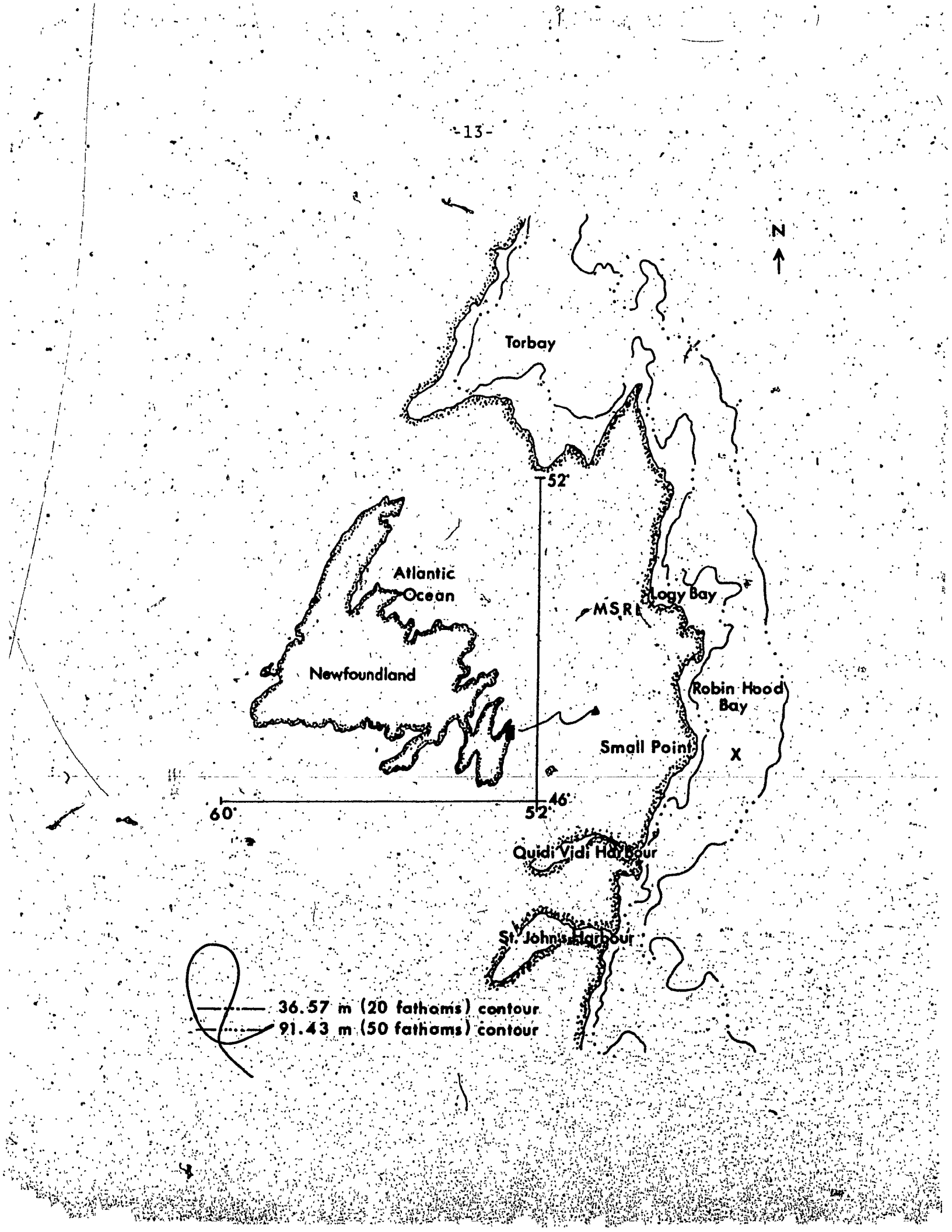
The field work was carried out in the vicinity of Small Point (47°36'N; 52°39'W), adjacent to Robin Hood Bay, on the east coast of the Avalon Peninsula, Newfoundland, and approximately 1.6 km. southeast of the Marine Sciences Research Laboratory (M.S.R.L.) of the Memorial University of Newfoundland (Fig. 1). Work was done in approximately 65 m. of water.

The sampling site was chosen for two reasons. First, it could be reached from Quidi Vidi Village, where the sampling boat was moored, in 15 to 20 minutes, so that samples could be returned to the laboratory and processed the same day. Second, this area had no fresh water run-off, and, because of the southward trend of the coastal current, was free of the pollution associated with the harbours of St. John's and Quidi Vidi.

2. Sampling Period

Sampling was carried out during seven trips from

Figure 1. Section of the east coast of the Avalon Peninsula showing the sampling station (X).


N
Torbay
52°
Atlantic
Ocean
Newfoundland
MSR
Logy Bay
Robin Hood
Bay
Small Point
X
60°
52°46'
Quidi Vidi Harbour
St. John's Harbour
36.57 m (20 fathoms) contour
91.43 m (50 fathoms) contour

November, 1972 to June, 1973. Although trips at two week intervals had been planned, bad weather, ice and mechanical difficulties with the sampling boat prevented a number of these from materializing. Field work was done from the M.S.R.L.'s launch, "Teal", except for the period from March 21 to May 29, 1973, when the research vessel "Beiner", of the College of Fisheries, Navigation, Marine Engineering and Electronics was used.

3. Measurements

On each trip, the following operations were carried out:

(a) Water depth determinations

The depth of water was measured with a sounding lead run over a meter wheel. A depth record was obtained using a bathythermograph (BT). The water depth value was used to determine the levels at which water samples were taken and plankton tows were made.

(b) Water temperature determinations.

Temperatures from surface to bottom were measured by means of a BT. A surface "bucket" temperature determination was also made and used to correct the BT trace, where necessary.

(c) Other measurements.

Air temperature was measured and observations were made on weather, wind force and direction, sea state and swell.

These data are given in Appendix 1.

4. Sampling Procedure

Samples were collected from three depths: approximately 1 m below the surface, approximately 1 m above the bottom, and middepth. When the presence of a thermocline was indicated upon examination of the BT trace, samples were taken at this level instead of middepth. The following samples were taken on each trip:

(a) Salinity samples.

Water samples for salinity determinations were collected from bottom and middepth (or thermocline) using a 1.2 liter capacity Nansen bottle and from the surface with a bucket. In the laboratory, specific gravities of the samples were measured and hydrometer readings converted to salinities using Seawater Temperature and Density Reduction Tables (Zerbe and Taylor, 1953).

(b) Plankton tow samples.

Plankton tows were made using a Clarke-Bumpus

sampler of 13 cm aperture, to which was attached a No. 20 nylon net (nominal mesh aperture of 0.076 mm). The operating depth of the sampler was determined from the wire angle (that angle made by the cable with the surface of the water) and the meter wheel reading, using a graph constructed for this purpose. A separate 15 minute tow was made at each depth.

Plankton material retained by the net was recovered by rinsing the outer surface of the net with the collecting cup attached, in a bucket of clean sea water. The cup was then carefully removed and its contents poured into a sterile jar. This rinsing procedure was carried out at least twice, after which the jar was capped tightly and iced.

B. Laboratory Work

1. (a) Culture Media.

For initial isolation of yeasts from the plankton samples collected, a selective medium had to be chosen which would inhibit bacterial populations and promote growth of yeast populations. It was decided to use the medium of Seshadri and Sieburth (1971).

All culture plates were poured a week prior to use and incubated at room temperature (20 - 22 C). They

were then critically examined for contamination using a stereoscopic binocular microscope. The sterile plates were then stored in a cold room to prevent excessive dehydration.

(b) Treatment of samples

Upon returning to the laboratory, the plankton samples were immediately transferred to a cold room (1.5 - 4.5C). All processing of the samples up to and including preparation of dilutions was done in a cold room.

Each plankton sample was thoroughly shaken for one minute after which one-half of the contents were poured into a sterile pre-chilled Waring blender cup and homogenized up for one minute. Ten ml were then removed, shaken thoroughly, and used to make dilutions of 1:10, 1:100 and 1:1000. Nine ml dilution blanks, prepared with paper-filtered (Whatman No. 5) natural sea water, were used. Being superfluous, the remaining half of each sample was discarded.

The samples were homogenized to enable dilutions to be more readily made and to break up clumps of micro-organisms in order to produce discrete colonies when cultured. (Moskovits, 1951; Seshadri and Sieburth, 1971).

With each of the dilutions prepared from each plankton sample, the membrane filtration procedure of Seshadri and Sieburth (1971) was carried out to help decrease any antifungal effects produced by the antibiosis of sea water. Two ml of a dilution were pipetted into the funnel of a membrane filtration apparatus, carrying a 47 mm Millipore filter of 0.45μ porosity. Twenty ml of sterile artificial sea water were then added to the sample and thoroughly mixed with a glass stirring rod. Sixteen ml of the diluted sample were pipetted off, leaving 6 ml above the filter. The latter volume was then partially removed as 2 ml aliquots, each of which was transferred to a separate membrane filtration apparatus for filtration and cultivation. The remaining 2 ml aliquot was then drawn through its filter. The membrane filters were then removed and placed on plates containing Seshadri and Sieburth's (1971) medium[1]. The plates were then incubated at 18C for 4 days.

At the end of the incubation period, Gram stains were made on the colonies. Twenty colonies were checked from Gram reaction from filters having ample populations.

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[1] The 2,3,5-triphenyltetrazolium chloride was omitted from this medium because it was not desirable to stain the colonies for the purposes of the present study.

If twenty or fewer colonies appeared on a filter, then ten, or all, if less than ten were present, were checked for Gram reaction.

Those colonies showing Gram positive, large, (1.5 - 8.5u wide x 3.5 - 28u long) ovoid cells were considered as being yeasts and were picked to yeast-malt extract broth (YM broth) (Wickerham, 1951). These were then successively streaked on yeast-malt extract agar (YM agar) (Wickerham, 1951) repicked and transferred to YM broth until pure cultures were obtained. The purified cultures were then transferred to YM agar slants containing a vitamin supplement[2] (Appendix II) on which they were maintained and stored in the cold room (1.5 - 4.5 C) after good growth had been established. Subculturing was carried out monthly.

2. Characterization Methods

Fresh 3-day cultures were prepared for all tests.

(a) Colony characteristics

Using Difco morphology agar, colony characteristics of the isolates, incubated at 25 C, were observed over a period of three weeks. The characteristics noted were:

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[2] The vitamin supplement was used in this medium to ensure the continuing viability of the cultures and to try to avoid mutations that may occur during prolonged storage. (Lodder, 1970).

colony diameter, form, elevation, surface, margin, consistency, density of growth and colour. Additionally, growth of the cultures in 2% glucose-yeast extract-peptone water was observed for "ring", surface film and pellicle formation.

(b) Cell morphology

Tubes of 2% glucose-yeast extract-peptone water and agar slants were inoculated with the isolates, incubated at 25C for 3 days and observed for growth. Wet mounts made from both the broth and the slants were stained with 1% methylene blue and examined for cell morphology. Measurements of cell dimensions were made on twenty cells from broths and twenty cells from slants for each isolate. The minimum and maximum measurements of width and length were recorded to give a dimension range for each isolate.

(c) Presence of mycelium or pseudomycelium

The presence of a mycelium or pseudomycelium and the form taken was examined using Wickerham's (1951) modification of the Dalmau procedure. Bacto-yeast morphology agar plates, allowed to dry at room temperature for 2 days, were used. Using a 3-day old slant culture, a single streak was made near one edge of the plate. Near the opposite edge of the plate, two point inoculations,

about 5 cm apart, were also made. Coverslips, sterilized by dipping in 95% ethanol and flaming, were placed, on cooling, over the center of the streak and over one of the point inoculations. The plates were then incubated at 25C for one week, after which observations were recorded.

A wet mount was made of cells from the edge of the aerobic colony and observations were made for cell arrangement and the presence of blastospores. The aerobic and anaerobic portions of the streak were examined, by the naked eye, for the presence of hyphae. These sections were then examined microscopically (10X and 40X oculars) to determine the presence of true mycelia with septae or pseudomycelia. The anaerobic point inoculation was then examined microscopically for the presence of hyphae. If there were no hyphae, blastospores were usually larger and more prevalent here than in the streak section.

(d) Ascospore formation

Cultures were examined for ascospore formation by incubating the isolates on YM agar at 25C for 3 days and then staining by Schaeffer and Fulton's (1933) method. If no ascospores were found, incubation was continued at room temperature for an additional 3 weeks, with a spore stain being carried out each week.

(3) Growth on Freshwater Media

All isolates were cultured in YM broth and on YM agar prepared with distilled water. Since all isolates grew well on such media, it was then possible to carry out the biochemical tests in freshwater media and avoid any possible adverse growth effects which might be exerted by the components of sea water.

3. Biochemical Testing

For identification of the isolates, the key developed by Barnett and Pankhurst (1974) was used. This is a computerized key based mainly on the reactions of yeasts to specific biochemical tests. The reactions are carried out in order of their ability to separate the organisms. Only fifty-two of the sixty-two suggested tests have this separating ability, although the remaining ten tests may have confirmatory value. The first fifty-two tests were carried out on all isolates. Only some of the remaining ten were carried out on certain of the organisms with the exception of D-glucose assimilation which was carried out on all isolates. All tests were done in duplicate. In some instances, it was not necessary to carry out all the tests in order to key out an organism, but, to save time, the tests were applied to all the cultures.

All media used in these tests are given in Appendix II and explained and described in Lodder (1970).

(a) Growth Measurements

Growth measurements were carried out for the following determinations: carbon assimilation test, nitrogen assimilation tests, vitamin-free medium growth test, cycloheximide resistance test, and 37 C growth test.

In carrying out these determinations, spectrophotometric measurements were made immediately following inoculation of the test medium and then weekly for a period of 3 to 5 weeks. A Bausch and Lomb Spectronic 20 spectrophotometer was used. Readings at 420mμ were recorded in terms of per cent transmission (%T).

Schultz and Pomper (1948) evaluated growth reactions by means of a spectrophotometer only, using the following scheme: 0 - 20% absorption = poor growth, 20 - 70% absorption = medium growth, 70 - 100% absorption = good growth. In my opinion, these groupings do not provide a very accurate method of evaulation of results because there exist too few groupings, each with too great a range making misinterpretation quite possible.

Ahearn (1964) estimated growth using optical

density (O.D.) readings according to the following scheme: 2.00 - 0.93 O.D. = optimal growth, 0.92 - 0.61 O.D. = good growth, 0.60 - 0.48 O.D. = weak growth, 0.47 - 0.19 O.D. = weak to negligible growth and 0.18 - 0.00 O.D. = negligible growth.

Wickerham and Burton (1948) described a method, equivalent to a growth measurement, estimating the turbidity of an inoculated medium. A number of India ink lines, each approximately 0.75 mm thick, were drawn on a white card. Each culture was shaken, then held against the lines on the card. Growth completely obliterating the lines was considered a 3+ reaction. If the lines appeared as an indistinct band, the growth reaction was considered to be 2+. Where the lines were distinguishable but with indistinct edges, the growth reaction was regarded as 1+. The absence of blurring of the lines was considered as a negative reaction. A 3+ or 2+ reaction was recorded as positive while a 1+ reaction was noted as a weak reaction.

In substituting a spectrophotometer for Wickerham and Burton's visual method of estimating growth, the following procedure was carried out.

Spectrophotometric readings from a number of cultures were recorded. Samples of these tubes, falling

within the following %T ranges were then chosen: 0-20, 20-40, 40-60, 60-80, 80-100. Growth in the tubes was then estimated using Wickerham's method. It was found that the black lines were obliterated in cultures which gave a %T of 30 or less. These were therefore considered 3+ reactions. The lines appeared as an indistinct band in cultures having a %T range of 30 to 60. These were considered 2+ reactions. The lines were distinguishable but had indistinct edges in cultures with a %T range of 60 to 70. These were considered 1+ reactions. Reactions in the range of 3+, 2+ and 1+ were called positive in the final tabulation of results. In cultures having merely a hazy appearance but with the lines completely visible, the %T range was from 70 to 80. These were considered to be + or questionable reactions. Cultures in which no growth occurred and through which the lines could be seen very distinctly, gave a %T of 80 to 100. These were considered negative reactions.

Spectrophotometric measurements were carried out in 13 X 100 mm disposable glass tubes. The uniformity of these tubes was such that one hundred, selected at random from several packages, showed 100% light transmission at 420mμ when filled with distilled water. Variance from this transmission occurred only when there were distinct flaws in the glass. Such tubes were discarded.

(1) Carbon assimilation tests

These tests were carried out to examine the ability of a yeast to utilize a given compound as a sole source of carbon. The following thirty-three substrates were tested: D-arabinose, L-arabinose, L-rhamnose, D-ribose, D-xylose (Sigma Chemicals), D-galactose (Difco), D-glucose (BDH Chemicals), methyl-α-D-glucoside, salicin, L-sorbose (Sigma Chemicals), cellobiose (Eastman Kodak), maltose (BDH Chemicals), lactose, melibiose, sucrose, trehalose, melezitose, raffinose, inulin, soluble starch, erythritol, ethanol, galactitol, D-gluicitol, glycerol, inositol, D-mannitol, ribitol, citric acid, 2-ketogluconic acid, glucono-δ-lactone, DL-lactic acid, and succinic acid (Sigma Chemicals). Compounds of the highest obtainable purity were used.

The concentration of each carbon source in solution was so adjusted as to give 0.2 g carbon. This would be equivalent to the concentration of carbon in a 0.5% solution of glucose. (Values for all compounds are given in Appendix II). With the exception of inulin and soluble starch, all solutions of carbon sources were sterilized by membrane filtration (0.45μ porosity). The inulin and starch solutions were autoclaved at 121C for 15 minutes (Lodder, 1970). When the carbon sources were acids or their salts, the pH of the medium was adjusted to 5.6 by the addition of sodium hydroxide pellets.

The carbon source substrates were added to Bacto-yeast nitrogen base medium (Difco) made up at 10X concentration and sterilized by membrane filtration (0.45μ porosity). Carbon source solutions and basal medium were stored separately in the refrigerator until ready for mixing and inoculation with the isolates[3]. Immediately prior to inoculation, 4.5 ml of each carbon source solution were added to 0.5 ml of basal medium in 13 X 100 mm disposable tubes with foam plug stoppers[4]. These tubes were each inoculated with one drop of a yeast isolate suspension using a Pasteur pipette and agitated for 30 seconds on a vortex mixer. The suspensions were prepared by transferring loopfuls of 3-day isolates grown on YM slants to 3 ml aliquots of sterile distilled water and agitating for 30 seconds on a vortex mixer.

Controls consisted of basal medium alone, inoculated with the isolates, together with uninoculated basal medium plus carbon source. The latter control was used to determine whether any deterioration of the reagents

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[3] As certain oligosaccharides may undergo slow hydrolysis in the basal medium during prolonged storage, carbon solutions and basal medium were filtered and stored separately, (Lodder, 1970).

[4] The foam plugs were thoroughly washed and then rinsed in distilled water to rid them of any traces of media or sugar solutions which could lead to erroneous results.

would occur over the prolonged incubation period. All experimental tubes and controls were incubated at 25C for 3 to 5 weeks. Observations were also made for the excretion of riboflavin into the medium. To test for production of starch, one drop of 0.02N iodine solution was added to each of the tubes used to detect glucose assimilation. The tubes were agitated on a vortex. Colours ranging from blue to green were denoted as positive for starch production.

(2) Nitrogen Assimilation Tests.

Two kinds of nitrogen assimilation tests were carried out. These were: nitrate assimilation and amino alkane assimilation.

(a) Nitrate Assimilation.

Bacto-yeast carbon base (Difco) was made up at 10X concentration and sterilized by membrane filtration. This medium was then added to 13 X 100 mm tubes in 0.5 ml amounts.

Filter-sterilized potassium nitrate solution (0.078g in 90 ml distilled water) was then added in 4.5 ml amounts to the tubes of carbon base medium and mixed thoroughly.

(b) Amino Alkane Assimilation

Filter-sterilized ethylamine hydrochloride solution

(0.064g in 90 ml distilled water) was added in 4.5 ml amounts to tubes of carbon base medium and mixed thoroughly.

For both these tests, the inoculation procedure was the same as that used for the carbon assimilation tests.

Because the cultures had been maintained on an enriched medium prior to the tests, the organisms would be able to carry over nitrogen to the experimental media giving erroneous results. To avoid such results, the following procedure was carried out for both types of nitrogen assimilation tests. The experimental media were inoculated with a drop of the culture suspension. This was incubated at 25C for one week. A drop of this growth was then inoculated into another tube of the same medium and incubated for 4 weeks with readings taken weekly.

(3) Vitamin-Free Medium Test.

This test was carried out using Bacto vitamin-free yeast base (Difco). This was made up at 10X concentration, sterilized by membrane filtration, and added in 0.5 ml amounts to 13 X 100 mm tubes containing 4.5 ml of sterile, distilled water. The tubes were inoculated with the isolates by the same method as used for the carbon assimilation tests. The tubes were incubated at 25C for one week. While growth may appear at the end of this period, this may be due to the carryover of vitamins from the enriched medium on which

the isolates were maintained. Therefore, as in the nitrogen assimilation tests, a second tube was inoculated with a drop of material from the first tube. The latter was then incubated for a period of 2 weeks and results were observed and recorded as for the carbon assimilation tests.

(4) 37C Growth Test

For this test, glucose assimilation medium was prepared and inoculated by the same method as used in the carbon assimilation tests. The tubes were incubated at 37C and observed for growth at 7 days and again at 24 days. The results were recorded as for the carbon assimilation tests.

(5) Cycloheximide resistance

A cycloheximide solution was prepared at a concentration of 0.01g in 90 ml distilled water and filter-sterilized. The antibiotic was added in 4.5 ml amounts to tubes containing 0.5 ml of Bacto-yeast nitrogen basal medium which also contained the same amount of glucose as used for the glucose assimilation test. The tubes were then incubated at 25C, shaken daily and observed weekly for 3 weeks. Results were recorded as for the carbon assimilation tests.

(6) Fermentation tests

A fermentation basal medium (Wickerham, 1951) was

prepared (Formula in Appendix II) and added in 2 ml aliquots to 13 X 100 mm tubes containing Durham tubes. The tubes were then autoclaved at 121C for 15 minutes.

The following eleven compounds were used: maltose, D-glucose, lactose, raffinose, sucrose, cellobiose, D-galactose, inulin, melibiose, trehalose and melezitose.

These compounds were prepared as 6% solutions in distilled water, sterilized by membrane filtration and added in 1 ml amounts to cooled fermentation basal medium.

These media were inoculated by the same method as for the carbon assimilation tests, incubated at 25C, shaken regularly, and observed for the production of acid and gas over a period of 24 days.

(7) 50% Glucose Growth Test

The medium was prepared by adding 50 g glucose to 50 ml of yeast infusion (Formula in Appendix II). Agar was then added to give a concentration of 3% (w/v) and dissolved using a hot water bath. Five ml aliquots were then dispensed into 20 X 125 mm screw-capped tubes and autoclaved at 121C for 15 minutes. The tubes were then slanted. The slants were inoculated by streaking with inoculum prepared in the same manner as for the carbon assimilation tests. Incubation was at 25C and observations of growth were made over a period of 4 weeks.

(8). Arbutin Growth Test

Arbutin agar was prepared by dissolving 0.5% arbutin and 2% agar in 100 ml yeast infusion. Aliquots of 5 ml were dispensed in 20 X 125 mm screw-capped tubes and autoclaved at 121C for 15 minutes. Immediately after sterilization, 2 - 3 drops of filter-sterilized 1% ferric ammonium citrate were added to each tube. The tubes were carefully agitated and then slanted.

Three-day isolates were streaked on the slants and the tubes were observed over a period of one week. If the organism had the ability to split arbutin, a brown colour developed in the medium.

(9) Gelatin Liquefaction

Ten g of gelatin were dissolved in 90 ml of hot distilled water. This solution was then added to 13 X 100 mm tubes in 4.5 ml amounts and autoclaved at 121C for 15 minutes. Upon cooling to approximately 37C, 0.5 ml aliquots of Bacto-yeast carbon base solution containing 5% glucose were added. The inoculum was prepared in the same manner as for the carbon assimilation tests, but deposited on the surface of the medium. Inoculated tubes were incubated at 25C and checked for liquefaction at 7 and 24 days after placing the tubes in the cold room (1.5 - 4.5 C) for a period of 30 minutes. The depth

of the liquefied gelatin, if present, was measured in millimeters. If the depth was 5 mm or more it was considered a positive reaction.

No investigation was carried out to evaluate or separate mating types.

RESULTS

The cultures were assigned numbers which provided certain basic information about the samples from which they were isolated. The first number (from 1 to 7) indicates the field trip and date of collection. The first letter indicates the type of sample from which the yeast was isolated. In this study, isolates were made only from plankton samples, so the letter is always "P". The second letter indicates the depth from which the sample was collected (S, surface; M, middepth; B, bottom). The following number indicates the dilution from which the isolate was obtained (usually, zero dilution, 0; sometimes a one in ten dilution, 10'). The last number indicates that the culture was one of a series taken from the original plate.

For example: 3PM10'3

3 - third field trip, February 6, 1973
P - plankton sample
M - middepth sample
10' - a one in ten dilution
3 - number 3 colony from the original plate.

The following yeasts were identified: *Debaryomyces hansenii*, *Rhodotorula rubra*, *Candida diddensii*, *Rhodotorula glutinus* var. *glutinus*, *Candida diffluens*, *Candida*

tropicalis, Rhodotorula graminis, Cryptococcus albidus var. albidus, Cryptococcus infirmo-miniatus, Candida humicola, Candida maritima, Leucosporidium scottii (as self-sporulating clones), Rhodotorula pallida and several "black yeasts". A list of the isolates identified is given in Table 1.

(a) Seasonal and Vertical Distribution

The seasonal and vertical distribution of the isolates is given in Tables 2 and 3. In the following discussion of the outcome of this study, the percentage which the isolates of a given species constitute is based on the occurrence of that species during the entire sampling period. Non-identifiable forms do not enter into percentage calculations.

Of all the isolates identified 29.3% were Debaryomyces hansenii. This species was found throughout the sampling period, but seemed most prevalent in samples from the second and third field trips (January 25 and February 6, 1973). Although identified from both middepth and bottom samples, 59.6% of the Debaryomyces hansenii isolates came from surface samples.

Rhodotorula rubra constituted 21.3% of the yeasts identified. For isolates of this species, 58.9% came from middepth samples but there were isolates identified from

TABLE 1

IDENTIFICATION OF ISOLATES

Isolate	Identification
1PS01	*Debaryomyces hansenii*
1PS02	*Debaryomyces hansenii*
1PS03	*Debaryomyces hansenii*
1PS04	*Debaryomyces hansenii*
1PS10'1	*Debaryomyces hansenii*
1PS10'4	*Candida diffluens*
1PS10'7	*Rhodotorula glutinis* var. *glutinis*
1PS10'9	*Candida diffluens*
1PM01	*Rhodotorula rubra*
1PM02	*Candida diffluens*
1PM03	*Rhodotorula glutinis* var. *glutinis*
1PM04	*Rhodotorula rubra*
1PM05	Unidentified
1PM06	*Rhodotorula rubra*
1PM07	*Candida diffluens*
1PM08	*Candida tropicalis*
1PM09	*Debaryomyces hansenii*
1PMQ11	*Candida diddensi*
1PM013	*Candida diffluens*
1PB06	*Rhodotorula rubra*
1PB07	*Debaryomyces hansenii*
1PB09	*Rhodotorula rubra*
1PB010	*Rhodotorula rubra*
2PS01	*Debaryomyces hansenii*
2PS02	"Black yeast"
2PS03	*Debaryomyces hansenii*
2PS04	*Debaryomyces hansenii*
2PS05	*Candida tropicalis*

TABLE 1 (CONTINUED)

Isolate	Identification
2PS06	*Debaryomyces hansenii*
2PS07	*Debaryomyces hansenii*
2PS08	*Debaryomyces hansenii*
2PS09	*Rhodotorula glutinis* var. *glutinis*
2PS010	*Cryptococcus infirmo-miniatus*
2PS011	*Rhodotorula glutinis* var. *glutinis*
2PS012	*Debaryomyces hansenii*
2PS013	*Candida diddensii*
2PS014	*Candida tropicalis*
2PS015	*Candida diddensii*
2PS016	*Rhodotorula graminis*
2PS017	*Candida tropicalis*
2PS10'2	*Cryptoccocus infirmo-miniatus*
2PS10'3	*Candida diddensii*
2PM01	*Candida diffluens*
2PM02	*Candida diddensii*
2PM03	*Candida diddensii*
2PM04	*Rhodotorula rubra*
2PM05	*Rhodotorula rubra*
2PM06	*Rhodotorula rubra*
2PM07	*Rhodotorula rubra*
2PM08	*Rhodotorula glutinis* var. *glutinis*
2PM011	*Rhodotorula rubra*
2PM012	*Rhodotorula rubra*
2PM013	*Candida diddensii*
2PM014	*Candida diddensii*
2PM015	*Rhodotorula rubra*
2PM016	*Candida diddensii*

TABLE 1 (CONTINUED)

Isolate	Identification
2PM10'1	*Rhodotorula rubra*
2PM10'2	Unidentified
2PB02	*Candida maritima*
2PB04	*Debaryomyces hansenii*
2PB05	*Rhodotorula glutinis* var. *glutinis*
2PB06	Unidentified
2PB07	*Rhodotorula graminis*
2PB08	Unidentified
2PB09	*Debaryomyces hansenii*
2PB010	*Candida tropicalis*
2PB012	*Debaryomyces hansenii*
2PB013	*Debaryomyces hansenii*
2PB014	*Debaryomyces hansenii*
2PB10'1	*Debaryomyces hansenii*
2PB10'2	*Candida humicola*
2PB10'11	*Debaryomyces hansenii*
2PB10'12	*Debaryomyces hansenii*
2PB10'13	*Candida humicola*
3PS01	*Rhodotorula rubra*
3PS02	*Candida maritima*
3PS03	*Debaryomyces hansenii*
3PS04	*Debaryomyces hansenii*
3PS05	*Debaryomyces hansenii*
3PS06	*Candida tropicalis*
3PS07	*Debaryomyces hansenii*
3PS08	*Debaryomyces hansenii*
3PS09	*Rhodotorula rubra*
3PS010	"Black yeast"
3PS011	*Debaryomyces hansenii*
3PS012	*Debaryomyces hansenii*

TABLE 1 (CONTINUED)

Isolate	Identification
3PS013	*Rhodotorula rubra*
3PS014	"Black yeast"
3PS015	*Rhodotorula rubra*
3PS016	*Rhodotorula graminis*
3PS017	*Rhodotorula glutinis* var. *glutinis*
3PS10'1	*Debaryomyces hansenii*
3PS10'2	*Rhodotorula graminis*
3PS10'9	Unidentified
3PM01	*Debaryomyces hansenii*
3PM02	*Rhodotorula rubra*
3PM03	*Rhodotorula rubra*
3PM04	*Rhodotorula rubra*
3PM05	Unidentified
3PM06	*Rhodotorula rubra*
3PM09	*Debaryomyces hansenii*
3PM010	*Rhodotorula glutinis* var. *glutinis*
3PM011	*Rhodotorula glutinis* var. *glutinis*
3PM012	*Rhodotorula glutinis* var. *glutinis*
3PM013	*Rhodotorula glutinis* var. *glutinis*
3PM014	*Rhodotorula rubra*
3PM015	Unidentified
3PM016	*Rhodotorula glutinis* var. *glutinis*
3PM10'3	Unidentified
3PB01	*Rhodotorula glutinis* var. *glutinis*
3PB02	*Rhodotorula rubra*
3PB03	*Candida diffluens*
3PB04	*Rhodotorula rubra*
3PB05	Unidentified
3PB06	*Candida diffluens*

TABLE 1 (CONTINUED)

Isolate	Identification
3PB07	*Debaryomyces hansenii*
3PB08	*Rhodotorula rubra*
3PB011	*Rhodotorula rubra*
3PB012	"Black yeast"
3PB013	*Rhodotorula glutinis* var. *glutinis*
3PB014	*Rhodotorula glutinis* var. *glutinis*
3PB015	*Candida tropicalis*
3PB016	Unidentified
3PB10'2	*Rhodotorula rubra*
4PS01	*Debaryomyces hansenii*
4PS02	*Debaryomyces hansenii*
4PS03	*Debaryomyces hansenii*
4PS05	*Candida diddensii*
4PS07	*Cryptococcus infirmo-miniatus*
4PS09	*Cryptococcus albidus* var. *albidus*
4PS010	*Candida diddensii*
4PS011	*Candida diddensii*
4PS012	*Rhodotorula graminis*
4PS013	*Rhodotorula rubra*
4PS014	*Rhodotorula rubra*
4PM01	*Rhodotorula glutinis* var. *glutinis*
4PM02	Unidentified
4PM03	*Candida diddensii*
4PM04	*Candida diddensii*
4PM05	*Cryptococcus albidus* var. *albidus*
4PM07	Unidentified
4PM010	*Cryptococcus albidus* var. *albidus*
4PM011	Unidentified
4PM012	*Rhodotorula rubra*

TABLE 1 (CONTINUED)

Isolate	Identification
4PM013	*Rhodotorula rubra*
4PM014	Unidentified
4PM015	*Rhodotorula rubra*
4PM016	*Cryptococcus albidus* var. *albidus*
4PB01	*Debaryomyces hansenii*
4PB02	Unidentified
4PB04	Unidentified
4PB05	Unidentified
4PB06	*Leucosporidium scottii*
4PB011	*Candida tropicalis*
4PB014	*Debaryomyces hansenii*
4PB015	Unidentified
5PS01	*Candida diddensii*
5PS02	*Candida diddensii*
5PS03	*Candida diddensii*
5PS04	*Candida diddensii*
5PS05	*Candida tropicalis*
5PS07	*Rhodotorula graminis*
5PS08	*Candida diddensii*
5PS010	*Candida diffluens*
5PS011	*Rhodotorula rubra*
5PM02	*Rhodotorula rubra*
5PM03	*Rhodotorula rubra*
5PM05	*Rhodotorula rubra*
5PM08	*Rhodotorula glutinis* var. *glutinis*
5PM015	*Rhodotorula pallida*
5PB02	Unidentified
5PB011	*Debaryomyces hansenii*
6PS02	Unidentified

TABLE 1 (CONTINUED)

Isolate	Identification
6PS03	*Debaryomyces hansenii*
6PS07	*Rhodotorula graminis*
6PS08	*Candida diddensii*
6PS09	*Candida diddensii*
6PS010	*Debaryomyces hansenii*
6PS011	*Candida diddensii*
6PS012	*Debaryomyces hansenii*
6PM01	*Debaryomyces hansenii*
6PM04	*Debaryomyces hansenii*
6PM06	*Candida diddensii*
6PM07	*Debaryomyces hansenii*
6PB01	*Candida humicola*
6PB02	*Debaryomyces hansenii*
6PB08	*Rhodotorula glutinis* var. *glutinis*
6PB015	*Rhodotorula rubra*
7PS01	Unidentified
7PS02	Unidentified
7PS03	*Candida diddensii*
7PS04	"Black yeast"
7PS05	*Debaryomyces hansenii*
7PS06	*Debaryomyces hansenii*
7PS08	*Candida diddensii*
7PS09	*Candida diddensii*
7PS010	"Black yeast"
7PS013	*Debaryomyces hansenii*
7PS10'1	*Debaryomyces hansenii*
7PS10'2	*Debaryomyces hansenii*
7PS10'3	Unidentified
7PM01	Unidentified

TABLE 1 (CONTINUED)

Isolate	Identification
7PM03	*Debaryomyces hansenii*
7PM05	*Rhodotorula rubra*
7PB01	*Rhodotorula glutinis* var. *glutinis*
7PB04	*Candida diffluens*
7PB07	*Leucosporidium scottii*
7PB010	Unidentified

TABLE 2

SEASONAL AND VERTICAL DISTRIBUTION OF ISOLATES

Species	Trip #1 23/11/72			Trip #2 25/1/73			Trip #3 6/2/73			Trip #4 21/3/73			Trip #5 15/5/73			Trip #6 29/5/73			Trip #7 13/6/73			Total
	S	M	B	S	M	B	S	M	B	S	M	B	S	M	B	S	M	B	S	M	B	
Debaryomyces hansenii	5	1	1	7	0	8	8	2	1	3	0	2	0	0	1	3	3	1	5	1	0	52
Rhodotorula rubra	0	3	3	0	8	0	4	5	5	2	3	0	1	3	0	0	0	1	0	1	0	39
Candida diddensii	0	1	0	3	5	0	0	0	0	3	2	0	4	0	0	3	1	0	3	0	0	25
Rhodotorula glutinis var. *glutinis*	1	1	0	2	1	1	1	5	3	0	1	0	0	1	0	0	0	1	0	0	1	19
Candida diffluens	2	3	0	0	1	0	0	0	2	0	0	0	2	0	0	0	0	0	0	0	1	11
Candida tropicalis	0	1	0	3	0	1	1	0	1	0	0	1	1	0	0	0	0	0	0	0	0	9
Rhodotorula graminis	0	0	0	1	0	1	2	0	0	1	0	0	1	0	0	1	0	0	0	0	0	7
Cryptococcus albidus var. *albidus*	0	0	0	0	0	0	0	0	0	1	3	0	0	0	0	0	0	0	0	0	0	4
Cryptococcus infirmo-miniatus	0	0	0	2	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	3
Candida humicola	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	3
Candida maritima	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
Leucosporidium scottii	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	2
Rhodotorula pallida	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1
"Black yeasts"	0	0	0	1	0	0	2	0	1	0	0	0	0	0	0	0	0	0	2	0	0	6
																						183

TABLE 3

NUMBER AND PERCENTAGE OF ISOLATES PER TRIP

	Trip #1	Trip #2	Trip #3	Trip #4	Trip #5	Trip #6	Trip #7	Total
Debaryomyces hansenii	7 (30.4)	15 (29.4)	11 (22.0)	5 (15.6)	1 (6.3)	7 (43.8)	6 (30.0)	52 (29.3)
Rhodotorula rubra	6 (26.1)	8 (15.7)	14 (28.0)	5 (15.6)	4 (25.0)	1 (6.3)	1 (5.0)	39 (21.33)
Candida diddensii	1 (4.3)	8 (15.7)	-	5 (15.6)	4 (25.0)	4 (25.0)	3 (15.0)	25 (13.7)
Rhodotorula glutinis var. *glutinis*	2 (8.7)	4 (7.8)	9 (18.0)	1 (3.1)	1 (6.3)	1 (6.3)	1 (5.0)	19 (10.4)
Candida diffluens	5 (21.7)	1 (1.9)	2 (4.0)	-	2 (12.5)	-	1 (5.0)	11 (6.0)
Candida tropicalis	1 (4.3)	4 (7.8)	2 (4.0)	1 (3.1)	1 (6.3)	-	-	9 (4.9)
Rhodotorula graminis	-	2 (3.9)	2 (4.0)	1 (3.1)	1 (6.3)	1 (6.3)	-	7 (3.8)
Cryptococcus albidus var. *albidus*	-	-	-	4 (12.5)	-	-	-	4 (2.2)
Cryptococcus infirmo-miniatus	-	2 (3.9)	-	1 (3.1)	-	-	-	3 (1.6)
Candida humicola	-	2 (3.9)	-	-	-	1 (6.3)	-	3 (1.6)
Candida maritima	-	1 (1.9)	1 (2.0)	-	-	-	-	2 (1.1)
Leucosporidium scottii	-	-	-	1 (3.1)	-	-	1 (5.0)	2 (1.1)
Rhodotorula pallida	-	-	-	-	1 (6.3)	-	-	1 (0.5)
"Black yeasts"	-	1 (1.9)	3 (6.0)	-	-	-	2 (10.0)	6 (3.3)
Unidentified	1 (4.3)	3 (5.8)	6 (12.0)	8 (25.0)	1 (6.3)	1 (6.3)	5 (25.0)	25 (12.0) of total isolates
Total Number of Isolates	25	51	50	32	16	16	20	208

[1] Numbers represent sums from all depths and percentages of the isolates are in parentheses.

all depths. The majority of *Rhodotorula rubra* isolates came from samples of the third field trip, February 6, 1973.

Candida diddensii comprised 13.7% of the yeasts identified. Most were isolated from samples of the second field trip on January 25, 1973. Sixty-four per cent of this species came from surface samples. No *Candida diddensii* were found in bottom samples.

Rhodotorula glutinis var. *glutinis* constituted 10.4% of the yeasts identified. The majority were isolated from the third field trip, February 6, 1973. Although identified from samples collected from all depths, 47.4% of this species came from middepth samples.

Of the species identified, 6.0% were *Candida diffluens*. The majority were obtained from samples collected during the first field trip on November 23, 1972. Surface and middepth samples seemed to contribute equally to the numbers of the species but a single isolate was obtained from a bottom sample.

Candida tropicalis comprised 4.9% of the species identified. Most were isolated from samples gathered on the second field trip, January 25, 1973. Of these yeasts, 55.6% were obtained from surface samples. However,

isolates were obtained from both middepth and bottom samples. *Candida tropicalis* species were not identified in samples collected from the sixth and seventh field trips (May 29 and June 13, 1973, respectively).

Rhodotorula graminis constituted 3.8% of the species identified. Most were found in the second and third field trips of January 25 and February 6, 1973, respectively. For this species, 85.7% were found in surface samples. One species was isolated from a bottom sample and none were isolated from middepth samples.

Of the species identified, 2.2% were *Cryptococcus albidus* var. *albidus*. All were isolated from the fourth field trip of March 21, 1973. Three of the isolates came from middepth samples and one came from a surface sample. No isolates came from bottom samples.

Both *Cryptococcus infirmo-miniatus* and *Candida humicola* constituted 1.6% of the yeasts identified. The former species came from surface samples of the second and fourth trips (January 25 and March 21, 1973, respectively). *Candida humicola* isolates were identified from bottom samples of the second and sixth trips (January 25 and May 29, respectively).

Both *Candida maritima* and *Leucosporidium scottii* constituted 1.1% of the yeasts identified. One isolate

each of the former came from a surface sample and a bottom sample on trips two and three (January 25 and February 6, 1973, respectively). *Leucosporidium scottii* was isolated from bottom samples on trips four and seven (March 21 and June 13, 1973, respectively).

Rhodotorula pallida was found to be the least common of all the yeasts identified, being present to the extent of 0.5%. The single isolate was obtained from a mid-depth sample on the fifth trip (May 15, 1973).

The "black yeasts" comprised 3.3% of the species identified. For these forms, 83.5% came from the surface samples. Though none were isolated from middepth samples, one was identified from a bottom sample. These "black yeasts" were isolated from samples collected on the second, third and seventh trips (January 23, February 6 and June 13, respectively).

(b) Cell morphology

Information on the cell morphology of the isolates is given in Table 4.

Most of the cells were long ovoid to ovoid to spherical. This is a very subjective evaluation giving no striking distinctions as diagnostic criteria. Cell

TABLE 4

CELL MORPHOLOGY

Isolate	Morphology of Cells	Dimensions (μ)		Presence of Mycelium or Pseudomycelium	Presence of Ascospores
		Width	Length		
1PS01	Ovoid	(5.0-6.4)	(4.0-8.0)	Absent	+, Spherical
1PS02	Ovoid	(2.6-6.0)	(3.2-7.9)	Absent	+, Spherical
1PS03	Ovoid	(2.0-5.3)	(3.0-8.0)	Absent	+, Spherical
1PS04	Ovoid	(2.4-6.0)	(3.5-6.8)	Absent	+, Spherical
1PS10'1	Ovoid	(2.0-5.5)	(3.0-6.4)	Absent	+, Spherical
1PS10'4	Ovoid	(3.4-4.0)	(5.9-8.6)	Pseudomycelia of branched chains of cylindrical cells.	-
1PS10'7	Ovoid	(2.7-4.6)	(5.0-8.3)	Absent	-
1PS10'9	Ovoid	(3.0-4.7)	(6.1-9.2)	Pseudomycelia of branched chains of elongate cylindrical cells.	-
1PM01	Ovoid	(3.0-5.0)	(5.0-8.7)	Absent	-
1PM02	Ovoid	(4.0-4.8)	(6.3-10.0)	Pseudomycelia of branched chains of cylindrical cells.	-
1PM03	Ovoid	(2.3-4.4)	(5.0-8.7)	Absent	-
1PM04	Ovoid	(2.4-4.6)	(4.7-8.1)	Absent	-
1PM05	Ovoid	(2.7-4.8)	(5.0-7.9)	Absent	-
1PM06	Ovoid	(3.0-4.9)	(5.0-7.9)	Absent	-
1PM07	Ovoid	(3.0-4.3)	(6.4-8.9)	Pseudomycelia of clumps of branched cylindrical cells.	-
1PM08	Ovoid to Spherical	(4.5-6.7)	(5.3-8.7)	Long, branching pseudomycelia with blastoconidia and blastospores.	-

TABLE 4 (CONTINUED)

Isolate	Morphology of Cells	Dimensions (μ)		Presence of Mycelium or Pseudomycelium	Presence of Ascospores
		Width	Length		
1PM09	Ovoid	(3.0-5.0)	(4.5-8.0)	Absent	+, Spherical
1PM011	Long-Ovoid	(1.7-3.4)	(5.9-9.1)	Long, branching pseudomycelia.	-
1PM013	Ovoid	(3.4-4.8)	(5.7-7.6)	Pseudomycelia of branched chains of cylindrical cells.	-
1PB06	Ovoid	(2.0-4.7)	(4.9-8.6)	Absent	-
1PB07	Ovoid to Spherical	(2.0-5.0)	(2.0-6.1)	Absent	+, Spherical
1PB09	Ovoid	(3.0-5.4)	(5.2-9.1)	Absent	-
1PB010	Ovoid	(2.9-5.1)	(5.1-8.6)	Absent	-
2PS01	Ovoid	(3.0-6.5)	(4.0-8.3)	Absent	+, Spherical
2PS02	Long-cylindrical	(3.0-11.2)	(5.6-21.0)	Mycelia with blastospores.	-
2PS03	Ovoid	(2.0-6.3)	(2.7-7.8)	Absent	+, Spherical
2PS04	Ovoid to Spherical	(3.0-5.6)	(3.0-6.9)	Absent	+, Spherical
2PS05	Ovoid	(4.0-7.1)	(6.5-10.1)	Long, branching pseudomycelia with blastoconidia and blastospores.	-
2PS06	Ovoid	(3.0-7.0)	(4.9-8.0)	Absent	+, Spherical
2PS07	Ovoid	(2.0-6.1)	(3.4-6.8)	Absent	+, Spherical
2PS08	Ovoid to Spherical	(2.0-4.8)	(2.6-5.0)	Absent	+, Spherical
2PS09	Ovoid	(2.6-3.9)	(4.7-6.4)	Absent	-
2PS010	Long-Ovoid	(2.5-3.2)	(6.1-10.0)	Absent	-
2PS011	Ovoid	(3.0-4.6)	(5.9-8.3)	Absent	-

TABLE 4 (CONTINUED)

Isolate	Morphology of Cells	Dimensions (μ)		Presence of Mycelium or Pseudomycelium	Presence of Ascospores
		Width	Length		
2PS012	Ovoid	(3.0-6.4)	(4.1-7.3)	Absent	+, Spherical
2PS013	Long-ovoid	(1.8-2.9)	(4.6-8.4)	Long, branching pseudomycelia with club-shaped tips.	-
2PS014	Ovoid to Spherical	(4.3-6.4)	(5.0-8.1)	Long, branching pseudomycelia with blastoconidia and blastospores.	-
2PS015	Ovoid to long-ovoid	(2.4-4.0)	(4.0-7.4)	Long, branching pseudomycelia.	-
2PS016	Ovoid	(3.0-3.8)	(4.6-6.0)	Absent	-
2PS017	Ovoid	(4.5-6.3)	(7.9-10.1)	Long, branching pseudomycelia, with blastoconidia and blastospores.	-
2PS10'2	Long-ovoid	(2.6-4.0)	(6.1-10.0)	Absent	-
2PS10'3	Long-ovoid	(1.9-3.6)	(4.8-9.4)	Long, branching pseudomycelia.	-
2PM01	Ovoid	(4.0-4.5)	(7.8-9.3)	Pseudomycelia of clumps of branched cylindrical cells.	-
2PM02	Long-ovoid	(2.0-3.1)	(5.0-8.9)	Long, branching pseudomycelia.	-
2PM03	Ovoid to long-ovoid	(2.1-3.4)	(4.0-7.9)	Long, branching pseudomycelia.	-
2PM04	Ovoid	(2.5-5.0)	(4.9-8.3)	Absent	-
2PM05	Ovoid	(2.9-4.9)	(5.0-7.4)	Absent	-
2PM06	Ovoid	(2.0-4.3)	(4.8-7.9)	Absent	-
2PM07	Ovoid	(2.7-5.5)	(5.3-9.2)	Absent	-
2PM08	Ovoid	(2.4-4.6)	(5.1-8.9)	Absent	-
2PM011	Ovoid	(2.0-4.6)	(4.5-7.1)	Absent	-

TABLE 4 (CONTINUED)

Isolate	Morphology of Cells	Dimensions (μ)		Presence of Mycelium or Pseudomycelium	Presence of Ascospores
		Width	Length		
2PMD12	Ovoid	(2.5-5.0)	(5.3-8.7)	Absent	-
2PMD13	Long-ovoid	(2.1-3.4)	(6.0-9.1)	Long, branching pseudomycelia with club-shaped tips.	-
2PMD14	Ovoid	(2.0-5.0)	(4.0-6.4)	Long, branching pseudomycelia.	-
2PMD15	Ovoid	(2.0-4.8)	(4.9-7.8)	Absent	-
2PMD16	Long-ovoid	(1.7-3.0)	(5.4-9.1)	Long, Branching pseudomycelia.	-
2PMD10'1	Ovoid	(2.5-5.3)	(5.4-8.8)	Absent	-
2PMD10'2	Ovoid	(3.0-4.8)	(5.2-7.9)	Absent	-
2PB02	Long-ovoid.	(2.0-4.8)	(3.1-8.4)	Pseudomycelia with blastoconidia and blastospores.	-
2PB04	Ovoid to spherical	(4.0-6.0)	(4.0-7.9)	Absent	+, Spherical
2PB05	Ovoid	(3.0-4.6)	(4.9-7.7).	Absent	-
2PB06	Ovoid to spherical	(4.0-5.3)	(4.4-6.9)	Absent	-
2PB07	Ovoid	(2.5-3.8)	(5.0-6.1)	Absent	-
2PB08	Long-ovoid	(3.1-4.7)	(7.9-10.1)	Long, branching pseudomycelia.	-
2PB09	Ovoid	(2.0-6.3)	(3.0-7.0)	Absent	+, Spherical
2PB010	Ovoid	(4.5-6.7)	(6.1-8.2)	Long, branching pseudomycelia with blastoconidia and blastospores.	-
2PB012	Ovoid	(3.0-6.4)	(4.0-7.2)	Absent	+, Spherical
2PB013	Ovoid	(3.0-5.8)	(3.4-7.4)	Absent	+, Spherical

TABLE 4 (CONTINUED).

Isolate	Morphology of Cells	Dimensions (μ)		Presence of Mycelium or Pseudomycelium	Presence of Ascospores
		Width	Length		
2PB014	Ovoid	(2.0-6.3)	(3.0-7.0)	Absent	+, Spherical
2PB10'1	Ovoid	(2.1-7.0)	(3.0-8.0)	Absent	+, Spherical
2PB10'2	Irregular, often ovoid to drop-like	(4.0-5.0)	(10.1-17.0)	Mycelia and pseudomycelia, long and branching.	-
2PB10'11	Ovoid to spherical	(3.1-6.5)	(3.3-7.0)	Absent	+, Spherical
2PB10'12	Ovoid	(2.2-6.7)	(3.0-7.4)	Absent	+, Spherical
2PB10'13	Irregular, ovoid to long-ovoid	(3.5-4.9)	(10.0-16.2)	Mycelia and pseudomycelia, long and branching.	-
3PS01	Ovoid	(2.4-5.0)	(5.1-9.1)	Absent	-
3PS02	Ovoid	(2.0-4.1)	(3.4-7.6)	Pseudomycelia with blastoconidia and blastospores.	-
3PS03	Ovoid	(2.0-6.4)	(3.9-7.3)	Absent	+, Spherical
3PS04	Ovoid to spherical	(2.0-4.3)	(2.1-4.8)	Absent	+, Spherical
3PS05	Ovoid	(3.1-7.0)	(3.7-7.5)	Absent	+, Spherical
3PS06	Ovoid to spherical	(4.7-6.3)	(5.9-8.1)	Long, branching pseudomycelia with blastospores.	-
3PS07	Ovoid	(2.0-7.0)	(3.0-7.6)	Absent	+, Spherical
3PS08	Ovoid	(2.1-6.2)	(3.4-8.0)	Absent	+, Spherical
3PS09	Ovoid	(2.6-5.2)	(5.0-8.9)	Absent	-
3PS010	Long-cylindrical	(3.0-9.5)	(6.2-17.1)	Mycelia with blastospores.	-
3PS011	Ovoid	(2.0-5.6)	(3.2-7.0)	Absent	+, Spherical

TABLE 4 (CONTINUED)

Isolate	Morphology of Cells	Dimensions (μ)		Presence of Mycelium or Pseudomycelium	Presence of Ascospores
		Width	Length		
3PS012	Ovoid	(2.4-7.0)	(3.0-7.7)	Absent	+, Spherical
3PS013	Ovoid	(2.5-5.5)	(6.1-9.4)	Absent	-
3PS014	Long-cylindrical	(4.0-9.3)	(7.1-14.4)	Mycelia with blastoconidia and blastospores.	-
3PS015	Ovoid	(2.0-4.1)	(4.7-8.0)	Absent	-
3PS016	Ovoid	(2.7-3.8)	(5.1-8.0)	Absent	-
3PS017	Ovoid	(2.9-4.6)	(5.9-7.3)	Absent	-
3PS10'1	Ovoid to spherical	(2.0-4.3)	(2.2-5.0)	Absent	+, Spherical
3PS10'2	Ovoid	(3.0-4.0)	(6.1-7.5)	Absent	-
3PS10'9	Ovoid	(2.6-3.8)	(5.4-6.9)	Absent	-
3PM01	Ovoid	(2.4-6.6)	(3.0-7.8)	Absent	+, Spherical
3PM02	Ovoid	(2.4-5.0)	(4.9-7.5)	Absent	-
3PM03	Ovoid	(2.0-5.4)	(5.0-8.4)	Absent	-
3PM04	Ovoid	(2.7-5.5)	(5.6-9.1)	Absent	-
3PM05	Long-ovoid	(3.1-4.6)	(6.7-9.1)	Long and branching pseudomycelia.	-
3PM06	Ovoid	(2.5-5.4)	(5.0-9.3)	Absent	-
3PM09	Ovoid	(2.0-6.1)	(3.0-7.0)	Absent	+, Spherical
3PM010	Ovoid	(2.4-4.6)	(4.7-6.8)	Absent	-
3PM011	Ovoid	(2.6-4.3)	(5.0-6.9)	Absent	-
3PM012	Ovoid	(3.0-4.1)	(5.0-7.0)	Absent	-

TABLE 4 (CONTINUED)

Isolate	Morphology of Cells	Dimensions (μ)		Presence of Mycelium or Pseudomycelium	Presence of Ascospores
		Width	Length		
3PM013	Ovoid	(2.6-4.0)	(5.6-7.4)	Absent	-
3PM014	Ovoid	(2.4-4.8)	(5.2-6.9)	Absent	-
3PM015	Ovoid	(1.9-3.6)	(5.1-6.4)	Absent	-
3PM016	Ovoid	(3.0-4.5)	(6.1-8.0)	Absent	-
3PM10'3	Ovoid	(4.5-5.6)	(8.1-10.2)	Long, branching pseudomycelia.	-
3PB01	Ovoid	(2.8-4.0)	(5.9-7.1)	Absent	-
3PB02	Ovoid	(2.0-4.6)	(4.8-7.3)	Absent	-
3PB03	Ovoid	(4.0-5.0)	(7.1-8.4)	Pseudomycelia of branches of chains of cylindrical cells.	-
3PB04	Ovoid	(2.1-4.9)	(5.1-8.3)	Absent	-
3PB05	Ovoid to spherical	(2.4-5.1)	(3.1-6.0)	Absent	-
3PB06	Ovoid	(3.8-4.6)	(7.4-9.0)	Pseudomycelia of branches of chains of cylindrical cells.	-
3PB07	Ovoid	(2.0-5.1)	(3.0-7.8)	Absent	+, Spherical
3PB08	Ovoid	(2.4-5.2)	(4.9-8.4)	Absent	-
3PB011	Ovoid	(2.0-4.6)	(5.0-7.8)	Absent	-
3PB012	Long-cylindrical	(3.1-10.4)	(7.8-23.1)	Mycelia with blastoconidia and blastospores.	-
3PB013	Ovoid	(2.3-4.3)	(5.2-6.6)	Absent	-
3PB014	Ovoid	(3.0-4.9)	(5.9-7.1)	Absent	-

TABLE 4 (CONTINUED)

Isolate	Morphology of Cells	Dimensions (μ)		Presence of Mycelium or Pseudomycelium	Presence of Ascospores
		Width	Length		
3PB015	Ovoid	(4.0-7.1)	(6.4-9.8)	Long, branching pseudomycelia with blastoconidia and blastospores.	-
3PB016	Ovoid	(3.1-4.4)	(6.4-8.8)	Absent	-
3PB10'2	Ovoid	(2.4-5.0)	(4.9-8.1)	Absent	-
4PS01	Ovoid	(2.1-4.3)	(3.0-7.1)	Absent	+, Spherical
4PS02	Ovoid to spherical	(2.0-3.4)	(2.4-6.8)	Absent	+, Spherical
4PS03	Ovoid	(2.0-6.4)	(3.1-7.0)	Absent	+, Spherical
4PS05	Long-ovoid	(1.8-3.0)	(5.4-9.1)	Long, branching pseudomycelia with club-shaped tips.	-
4PS07	Long-ovoid	(3.0-4.2)	(7.8-12.0)	Absent	-
4PS09	Ovoid to spherical	(3.4-6.9)	(6.0-7.1)	Absent	-
4PS010	Ovoid	(1.9-3.5)	(4.0-5.8)	Long, branching pseudomycelia.	-
4PS011	Long-ovoid	(2.0-3.1)	(5.9-8.7)	Long, branch pseudomycelia.	-
4PS012	Ovoid	(2.5-3.7)	(6.0-7.6)	Absent	-
4PS013	Ovoid	(2.6-5.5)	(5.0-9.1)	Absent	-
4PS014	Ovoid	(2.3-5.0)	(4.7-8.6)	Absent	-
4PM01	Ovoid	(2.4-4.3)	(5.0-7.1)	Absent	-
4PM02	Ovoid to spherical	(1.9-3.0)	(4.1-4.9)	Absent	-
4PM03	Ovoid	(2.0-3.2)	(4.1-6.0)	Long, branching pseudomycelia.	-
4PM04	Long-ovoid	(1.7-2.9)	(5.0-7.2)	Long, branching pseudomycelia.	-

TABLE 4 (CONTINUED)

Isolate	Morphology of Cells	Dimensions (μ)		Presence of Mycelium or Pseudomycelium	Presence of Ascospores
		Width	Length		
4PM05	Ovoid	(3.5-7.0)	(4.9-8.1)	Absent	-
4PM07	Long-ovoid	(2.0-4.8)	(8.9-11.2)	Long, branching pseudomycelia.	-
4PM010	Ovoid	(3.8-6.9)	(5.0-8.6)	Absent	-
4PM011	Ovoid	(2.1-4.0)	(4.4-6.1)	Absent	-
4PM012	Ovoid	(2.4-5.0)	(4.8-7.9)	Absent	-
4PM013	Ovoid	(2.0-4.7)	(5.0-8.0)	Absent	-
4PM014	Long-ovoid	(1.9-3.1)	(5.7-9.3)	Long, branching pseudomycelia.	-
4PM015	Ovoid	(2.3-4.9)	(5.0-7.8)	Absent	-
4PM016	Ovoid to spherical	(3.5-6.0)	(5.0-6.5)	Absent	-
4PB01	Ovoid	(2.0-5.4)	(2.9-7.3)	Absent	+, Spherical
4PB02	Ovoid	(3.1-4.8)	(5.9-7.4)	Absent	-
4PB04	Long-ovoid	(2.1-4.0)	(6.3-9.8)	Long, branching pseudomycelia.	-
4PB05	Ovoid	(2.4-5.0)	(4.1-7.2)	Absent	-
4PB06	Long-ovoid	(1.7-4.3)	(5.1-12.6)	Mycelia and pseudomycelia with blastoconidia. Teliospores.	-
4PB011	Ovoid to spherical	(4.3-6.2)	(5.9-9.3)	Long, branching pseudomycelia with blastoconidia and blastospores.	-
4PB014	Ovoid	(2.1-4.3)	(3.3-7.0)	Absent	-
4PB015	Ovoid	(3.0-4.1)	(5.9-7.2)	Absent	-
5PS01	Long-ovoid	(2.0-4.0)	(6.1-9.4)	Long, branching pseudomycelia with club-shaped tips.	-

TABLE 4 (CONTINUED)

Isolate	Morphology of Cells	Dimensions (μ)		Presence of Mycelium or Pseudomycelium	Presence of Ascospores
		Width	Length		
5PS02	Ovoid	(4.0-4.9)	(7.9-10.1)	Pseudomycelia of clumps of branching cylindrical cells.	-
5PS03	Long-ovoid	(1.9-3.4)	(5.8-8.7)	Long, branching pseudomycelia with club-shaped tips.	-
5PS04					
5PS05	Ovoid	(4.7-8.0)	(7.1-10.8)	Long, branching pseudomycelia with blastospores.	-
5PS07	Ovoid	(2.5-4.1)	(4.8-7.8)	Absent	-
5PS08	Long-ovoid	(2.0-2.9)	(6.1-7.3)	Long, branching pseudomycelia with club-shaped tips.	-
5PS010	Ovoid	(3.0-4.9)	(7.1-8.9)	Pseudomycelia of branched chains of cylindrical cells.	-
5PS011	Ovoid	(2.0-4.8)	(5.0-7.6)	Absent	-
5PM02	Ovoid	(2.3-5.3)	(5.1-8.7)	Absent	-
5PM03	Ovoid	(2.0-4.9)	(5.2-8.5)	Absent	-
5PM05	Ovoid	(2.2-5.0)	(5.4-8.4)	Absent	-
5PM08	Ovoid	(2.3-5.0)	(5.6-8.1)	Absent	-
5PM015	Ovoid to spherical	(3.0-4.1)	(4.6-7.0)	Absent	-
5PB02	Ovoid	(2.3-5.0)	(6.1-8.2)	Absent	-
5PB011	Ovoid	(2.4-7.0)	(4.1-8.0)	Absent	+, Spherical
6PS02	Ovoid	(2.0-4.8)	(6.3-7.8)	Absent	-
6PS03	Ovoid	(2.0-4.7)	(3.1-6.8)	Absent	+, Spherical
6PS07	Ovoid	(3.0-4.3)	(5.1-7.7)	Absent	-

TABLE 4 (CONTINUED)

Isolate	Morphology of Cells	Dimensions (μ)		Presence of Mycelium or Pseudomycelium	Presence of Ascospores
		Width	Length		
6PS08	Long-ovoid	(2.0-3.1)	(5.8-8.7)	Long, branching pseudomycelia with club-shaped tips.	-
6PS09	Ovoid	(2.1-4.0)	(4.4-5.6)	Long, branch pseudomycelia with club-shaped tips.	-
6PS010	Ovoid	(2.1-6.4)	(3.0-7.1)	Absent	+, Spherical
6PS011	Long-ovoid	(1.8-3.0)	(5.8-8.6)	Long, branching pseudomycelia.	-
6PS012	Ovoid	(2.0-4.4)	(2.9-6.8)	Absent	+, Spherical
6PM01	Ovoid	(2.9-6.1)	(3.7-7.4)	Absent	+, Spherical
6PM04	Ovoid to spherical	(2.5-3.7)	(2.8-4.1)	Absent	+, Spherical
6PM06	Long-ovoid	(2.0-3.1)	(5.5-8.9)	Long, branching pseudomycelia.	-
6PM07	Ovoid	(3.0-7.1)	(3.7-8.0)	Absent	+, Spherical
6PB01	Irregular, ovoid	(4.0-4.7)	(11.2-14.7)	Mycelia and pseudomycelia long and branching.	-
6PB02	Ovoid	(2.0-5.6)	(3.0-7.9)	Absent	+, Spherical
6PB08	Ovoid	(3.0-4.8)	(6.1-8.0)	Absent	-
6PB015	Ovoid	(2.3-5.1)	(4.9-7.9)	Absent	-
7PS01	Ovoid	(2.0-4.6)	(5.5-8.3)	Absent	+, Spherical
7PS02	Ovoid to spherical	(3.1-4.2)	(4.4-5.3)	Absent	-
7PS03	Long-ovoid	(1.6-2.4)	(5.7-8.9)	Long, branching pseudomycelia.	-
7PS04	Long-cylindrical	(3.0-10.1)	(8.1-17.3)	Mycelia with blastoconidia and blastospores.	-

TABLE 4 (CONTINUED)

Isolate	Morphology of Cells	Dimensions (μ)		Presence of Mycelium or Pseudomycelium	Presence of Ascospores
		Width	Length		
7PS05	Ovoid	(2.3-6.1)	(3.0-7.5)	Absent	+, Spherical
7PS06	Ovoid to spherical	(2.0-4.0)	(2.3-5.9)	Absent	+, Spherical
7PS08	Long-ovoid	(1.8-2.7)	(5.8-8.4)	Long, branching pseudomycelia.	-
7PS09	Long-ovoid	(2.0-3.1)	(6.0-8.6)	Long, branching pseudomycelia.	-
7PS010	Long-cylindrical	(4.0-8.9)	(10.3-17.7)	Mycelia with blastocondidia and blastospores.	-
7PS013	Ovoid	(3.0-6.4)	(4.1-7.0)	Absent	+, Spherical
7PS10'1	Ovoid to spherical	(2.4-5.3)	(2.8-6.7)	Absent	+, Spherical
7PS10'2	Ovoid	(2.0-6.0)	(4.3-8.0)	Absent	+, Spherical
7PS10'3	Ovoid	(3.1-5.3)	(6.1-8.3)	Absent	-
7PM01	Ovoid to spherical	(2.1-3.0)	(4.1-5.0)	Absent	-
7PM03	Ovoid	(2.0-5.3)	(4.1-6.9)	Absent	+, Spherical
7PM05	Ovoid	(2.5-5.4)	(5.1-9.1)	Absent	-
7PB01	Ovoid	(2.5-4.0)	(5.9-7.6)	Absent	-
7PB04	Ovoid	(3.6-4.8)	(7.9-9.3)	Pseudomycelia of branches of chains of cylindrical cells.	-
7PB07	Long-ovoid	(2.0-4.3)	(6.1-13.4)	Mycelia and pseudomycelia with blastoconidia. Teliospores.	-
7PB010	Long-ovoid	(1.6-3.1)	(7.8-10.4)	Long, branching pseudomycelia.	-

form and cell dimensions can be of some use in identification, but only after all other data have been evaluated.

The presence of a mycelium or pseudomycelium can be of great diagnostic significance. In this study, the only isolates with mycelia were the two cultures of *Leucosporidium scottii*, the three cultures of *Candida humicola* and the six "black yeasts".

The type of pseudomycelium, if present, is also important and includes the formation of club-shaped tips, blastoconidia and blastospores, or the formation of the characteristic clamp connections of *Leucosporidium scottii*.

This information plus the presence or absence of ascospores give us a good indication of the method of reproduction of the organism. For example: in this study, all cultures with long, branching pseudomycelia with club-shaped tips or slight variations of this and the absence of ascospores keyed out as *Candida diddensii*. On the other hand, *Debaryomyces hansenii* formed no mycelia or pseudomycelia, but did present spherical ascospores. The two cultures of *Leucosporidium scottii* produced very thick-walled spores, called teliospores placing this species in the order Ustilaginales. One unidentified culture produced ascospores. The rest of

the ascospore-producing cultures keyed out to be *Debaryomyces hansenii*.

It needs to be emphasized that the presence or absence of the various structures mentioned is useful for identification purposes only in addition to the biochemical characteristics.

(c) Colony characteristics

Colony characteristics are given in Table 5. These characteristics are also useful after other data have been evaluated.

The colours of the colonies can be quite distinctive as in the black colour of the "black yeasts" due to the presence of melanin pigment. The reds and corals of *Rhodotorula* and *Cryptococcus* species are characteristic for these yeasts and are due to the presence of carotenoid pigments.

Regarding the consistency of the colony, the tough, viscous nature of *Candida tropicalis* is quite distinctive. The mucoid to slimy consistency of *Cryptococcus albidus* var. *albidus* is characteristic of this species. The wrinkled and hairy surfaces of the "black yeasts" are characteristic of these isolates. *Candida diffluens*

TABLE 5

COLONY CHARACTERISTICS

Isolate	2% Glucose-Yeast Extract-Peptone Water	Density of Growth *	Colony Diameter (mm)	Form	Surface	Elevation	Margin	Consistency	Colour
1PS01	Ring	+ + + Opaque	2 - 40	Circular	Smooth, glossy	Flat	Entire	Butyrous	Greyish-white
1PS02	Ring	+ + + Opaque	4 - 23	Circular	Smooth, glossy	Flat	Entire	Butyrous	Greyish-white
1PS03	Ring	+ + + Opaque	2 - 31	Circular	Slightly wrinkled,glossy	Slightly raised	Entire with a few indentations	Butyrous	Greyish-yellow
1PS04	Ring	+ + + Opaque	3 - 17	Circular	Smooth, glossy	Flat	Entire	Butyrous	Greyish-white
1PS10'1	Ring	+ + + Opaque	4 - 34	Circular	Smooth, glossy	Flat	Entire	Butyrous	Greyish-white
1PS10'4	Ring	+ + + Opaque	3 - 10	Circular	Smooth, glossy	Umbonate	Lobate	Mucoid	Brownish
1PS10'7	Thin Ring	+ + Opaque	3 - 9	Circular	Smooth, glossy	Low convex	Entire	Mucoid	Reddish-orange
1PS10'9	Ring	+ + + Opaque	2 - 14	Irregular	Smooth, glossy	Umbonate	Lobate	Mucoid	Brownish
1PM01	Thin Ring and surface film	+ + + Opaque	4 - 16	Irregular	Smooth, glossy	Low convex	Entire	Mucoid	Coral
1PM02	Ring	+ + + Opaque	3 - 10	Circular	Smooth, glossy	Umbonate	Lobate	Mucoid	Brownish
1PM03	Thin Ring	+ + Opaque	4 - 21	Irregular	Wrinkled,glossy	Low convex	Lobate	Pasty	Orange
1PM04	Ring and surface film	+ + + Opaque	3 - 21	Irregular	Smooth, glossy	Low convex	Entire	Mucoid	Coral-red
1PM05	Ring	+ + + Opaque	2 - 11	Irregular	Smooth, glossy	Flat	Entire	Butyrous	Creamy
1PM06	Surface film	+ + Opaque	7 - 21	Irregular	Smooth, glossy	Flat	Entire	Mucoid	Coral-red
1PM07	Ring	+ + Opaque	6 - 14	Irregular	Ridges in center, glossy	Umbonate	Lobate	Mucoid	Brownish
1PM08	Ring	+ + + Opaque	3 - 20	Irregular	Wrinkled,hairy	Slightly raised	Lobate	Tough	Whitish-cream

* + = scant growth, ++ = moderate growth, +++ = good growth

TABLE 5 (CONTINUED)

Isolate	2% Glucose-Yeast Extract-Peptone Water	Density of Growth	Colony Diameter (mm)	Form	Surface	Elevation	Margin	Consistency	Colour
1PM09	Ring	+ + + Opaque	2 - 36	Circular	Smooth,glossy	Flat	Entire	Butyrous	Greyish-white
1PM011	Ring	+ + + Opaque	3 - 15	Irregular	Smooth,glossy	Flat	Lobate	Butyrous	Creamy
1PM013	Ring	+ + + Opaque	4 - 10	Circular	Ridges in center,glossy	Umbonate	Lobate	Mucoid	Creamy-brown
1PB06	Thin ring	+ + Opaque	4 - 13	Circular	Smooth,glossy	Low convex	Entire	Mucoid	Pink
1PB07	Ring	+ + + Opaque	2 - 14	Circular	Slightly wrinkled,dull	Slightly raised	Entire with a few indentations	Butyrous	Yellowish
1PB09	Thin ring	+ + Opaque	3 - 17	Irregular	Smooth,glossy	Flat	Entire	Mucoid	Pink
1PB010	Thin ring	+ + Opaque	4 - 18	Irregular	Smooth,glossy	Low convex	Entire	Mucoid	Pink
2PS01	Ring	+ + + Opaque	3 - 21	Circular	Smooth,glossy	Flat	Entire	Butyrous	Greyish-white
2PS02	Pellicle	+ + + Opaque	4 - 18	Irregular	Wrinkled,hairy	Raised	Lobate	Tough	Black
2PS03	Broken pellicle	+ + + Opaque	4 - 39	Circular	Smooth,glossy	Flat	Entire	Butyrous	Greyish-white
2PS04	Ring	+ + + Opaque	2 - 18	Circular	Slightly wrinkled, dull	Slightly raised	Entire with a few indentations	Butyrous	Yellowish
2PS05	Ring	+ + + Opaque	3 - 10	Circular	Wrinkled,hairy	Slightly raised	Lobate	Tough	Whitish-cream
2PS06	Ring	+ + + Opaque	3 - 38	Circular	Smooth,glossy	Flat	Entire	Butyrous	Greyish-white
2PS07	Ring	+ + + Opaque	2 - 25	Circular	Smooth,glossy	Flat	Entire	Butyrous	Greyish-white
2PS08	Broken pellicle	+ + + Opaque	3 - 31	Circular	Slightly wrinkled,dull	Slightly raised	Entire with a few indentations	Butyrous	Yellowish

TABLE 5 (CONTINUED)

Isolate	2% Glucose-Yeast Extract-Peptone Water	Density of Growth	Colony Diameter (mm)	Form	Surface	Elevation	Margin	Consistency	Colour
2PS09	Thin ring	+ + Opaque	2 - 17	Irregular	Wrinkled,glossy	Low convex	Lobate	Mucoid	Orange
2PS010	Ring	+ + + Opaque	3 - 14	Circular	Smooth,glossy	Convex	Entire	Butyrous	Red
2PS011	Thin ring	+ + Opaque	4 - 13	Irregular	Smooth,glossy	Low convex	Lobate	Mucoid	Reddish-orange
2PS012	Ring	+ + + Opaque	2 - 29	Circular	Smooth,glossy	Flat	Entire	Butyrous	Greyish-white
2PS013	Broken pellicle	+ + + Opaque	3 - 14	Irregular	Wrinkled, semi-dull	Slightly raised	Lobate	Butyrous	Creamy
2PS014	Ring	+ + Opaque	4 - 10	Irregular	Wrinkled,dull	Slightly raised	Lobate	Tough	Whitish-cream
2PS015	Ring	+ + Opaque	4 - 17	Irregular	Wrinkled,dull	Slightly raised	Lobate	Butyrous	Creamy
2PS016	Ring	+ + Opaque	3 - 20	Irregular	Smooth,glossy	Flat	Lobate	Mucoid	Coral Red
2PS017	Ring	+ + + Opaque	3 - 16	Irregular	Smooth, dull	Slightly raised	Lobate	Tough	Whitish-cream
2PS10'2	Ring	+ + + Opaque	2 - 10	Circular	Smooth,glossy	Convex	Entire	Butyrous	Red
2PS10'3	Ring	+ + + Opaque	7 - 23	Irregular	Wrinkled,dull	Slightly raised	Lobate	Pasty	Creamy
2PM01	Ring	+ + + Opaque	3 - 14	Irregular	Ridges in center,glossy	Umbonate	Lobate	Mucoid	Brownish
2PM02	Broken pellicle	+ + + Opaque	3 - 11	Circular	Smooth,glossy	Slightly raised	Lobate	Butyrous	Creamy
2PM03	Ring	+ + Opaque	4 - 16	Irregular	Smooth,glossy	Slightly raised	Lobate	Butyrous	Creamy

TABLE 5 (CONTINUED)

Isolate	2% Glucose-Yeast Extract-Peptone Water	Density of Growth	Colony Diameter (mm)	Form	Surface	Elevation	Margin	Consistency	Colour
2PM04	Thin ring and surface film	+ + + Opaque	4 - 11	Irregular	Smooth,glossy	Low convex	Entire	Mucoid	Coral
2PM05	Thin ring	+ + Opaque	3 - 20	Irregular	Smooth,glossy	Low convex	Entire	Mucoid	Coral
2PM06	Thin ring	+ + Opaque	4 - 18	Irregular	Smooth,glossy	Flat	Entire	Mucoid	Pink
2PM07	Thin ring and surface film	+ + + Opaque	3 - 19	Irregular	Smooth,glossy	Low convex	Entire	Mucoid	Coral
2PM08	Thin ring	+ + Opaque	2 - 8	Circular	Smooth,glossy	Low convex	Lobate	Mucoid	Orange
2PM011	Thin ring and surface film	+ + + Opaque	4 - 20	Irregular	Smooth;glossy	Low convex	Entire	Mucoid	Coral-red
2PM012	Thin ring	+ + Opaque	4 - 17	Circular	Smooth,glossy	Flat	Entire	Mucoid	Pink
2PM013	Broken pellicle	+ + + Opaque	3 - 14	Irregular	Wrinkled, simi-dull	Slightly raised	Lobate	Pasty	Creamy
2PM014	Ring	+ + Opaque	2 - 13	Irregular	Smooth,glossy	Low convex	Lobate	Butyrous	Greyish-cream
2PM015	Thin ring and surface film	+ + + Opaque	3 - 31	Irregular	Smooth,glossy	Low convex	Entire	Mucoid	Coral
2PM016	Broken pellicle	+ + + Opaque	2 - 11	Irregular	Smooth,glossy	Slightly raised	Lobate	Butyrous	Creamy
2PM10'1	Thin ring	+ + Opaque	4 - 17	Circular	Smooth,glossy	Flat	Entire	Mucoid	Pink
2PM10'2	Ring	+ + Opaque	3 - 14	Circular	Wrinkled, semi-dull	Slightly raised	Lobate	Mucoid	Whitish-cream
2PB02	Thin ring	+ + Opaque	2 - 10	Irregular	Smooth,glossy	Slightly raised	Lobate	Mucoid	Greyish-cream

TABLE 5 (CONTINUED)

Isolate	2% Glucose-Yeast Extract-Peptone Water	Density of Growth	Colony Diameter (mm)	Form	Surface	Elevation	Margin	Consistency	Colour
2PB04	Ring	+ + + Opaque	2 - 19	Circular	Smooth, glossy	Flat	Entire	Butyrous	Greyish-white
2PB05	Thin ring	+ + Opaque	3 - 14	Irregular	Wrinkled, glossy	Slightly raised	Lobate	Pasty	Orange
2PB06	Ring	+ + Opaque	5 - 16	Irregular	Smooth, glossy	Slightly raised	Lobate	Butyrous	Creamy
2PB07	Ring and surface film	+ + + Opaque	4 - 21	Irregular	Wrinkled, glossy	Low convex	Lobate	Mucoid	Coral red
2PB08	Ring	+ + + Opaque	2 - 9	Circular	Smooth, glossy	Low convex	Entire	Butyrous	Creamy
2PB09	Broken pellicle	+ + + Opaque	3 - 32	Circular	Slightly wrinkled, dull	Slightly raised	Entire with a few indentations	Butyrous	Yellowish
2PB010	Ring	+ + + Opaque	3 - 14	Irregular	Wrinkled, hairy	Slightly raised	Lobate	Tough	Whitish-cream
2PB012	Ring	+ + + Opaque	3 - 19	Circular	Smooth, glossy	Flat	Entire	Butyrous	Greyish-white
2PB013	Ring	+ + + Opaque	2 - 31	Circular	Smooth, glossy	Flat	Entire	Butyrous	Greyish-white
2PB014	Ring	+ + + Opaque	4 - 42	Circular	Smooth, glossy	Flat	Entire	Butyrous	Greyish-white
2PB10'1	Broken pellicle	+ + + Opaque	2 - 23	Circular	Slightly wrinkled, dull	Slightly raised	Entire with a few indentations	Butyrous	Yellowish
2PB10'2	Pellicle	+ + + Opaque	3 - 14	Irregular	Smooth, glossy	Flat	Entire	Mucoid	Yellow
2PB10'11	Broken pellicle	+ + + Opaque	3 - 17	Circular	Slightly wrinkled, dull	Slightly raised	Entire with a few indentations	Butyrous	Yellowish

TABLE 5 (CONTINUED)

Isolate	2% Glucose-Yeast Extract-Peptone Water	Density of Growth	Colony Diameter (mm)	Form	Surface	Elevation	Margin	Consistency	Colour
2PB10'12	Ring	+ + + Opaque	2 - 24	Circular	Smooth,glossy	Flat	Entire	Butyrous	Greyish-white
2PB10'13	Pellicle	+ + + Opaque	4 - 18	Irregular	Smooth,glossy	Flat	Entire	Mucoid	Yellow
3PS01	Thin ring	+ + Opaque	3 - 14	Circular	Smooth,glossy	Flat	Entire	Mucoid	Pink
3PS02	Thin ring	+ + + Opaque	4 - 16	Irregular	Wrinkled, semi-dull	Slightly raised	Lobate	Mucoid	Greyish-cream
3PS03	Ring	+ + + Opaque	2 - 17	Circular	Smooth,glossy	Flat	Entire	Butyrous	Greyish-white
3PS04	Ring	+ + + Opaque	3 - 24	Circular	Smooth,glossy	Flat	Entire	Butyrous	Greyish-white
3PS05	Broken pellicle	+ + + Opaque	2 - 27	Circular	Slightly wrinkled,dull	Slightly raised	Entire with a few indentations	Butyrous	Yellowish
3PS06	Ring	+ + + Opaque	3 - 10	Irregular	Wrinkled,hairy	Slightly raised	Lobate	Viscid	Whitish-cream
3PS07	Broken pellicle	+ + + Opaque	4 - 17	Circular	Slightly wrinkled, dull	Slightly raised	Entire with a few indentations	Butyrous	Yellowish
3PS08	Broken pellicle	+ + + Opaque	3 - 19	Circular	Wrinkled, dull	Slightly raised	Entire with a few indentations	Butyrous	Greyish-yellow
3PS09	Ring and surface film	+ + + Opaque	4 - 28	Irregular	Smooth, glossy	Low convex	Entire	Mucoid	Coral
3PS010	Pellicle	+ + + Opaque	3 - 14	Irregular	Wrinkled,hairy	Raised	Lobate	Tough	Black
3PS011	Ring	+ + + Opaque	2 - 11	Circular	Smooth,glossy	Flat	Entire	Butyrous	Greyish-white

TABLE 5 (CONTINUED)

Isolate	2% Glucose-Yeast Extract-Peptone Water	Density of Growth	Colony Diameter (mm)	Form	Surface	Elevation	Margin	Consistency	Colour
3PS012	Ring	+ + Opaque	2 - 10	Circular	Smooth, glossy	Flat	Entire	Butyrous	Greyish-white
3PS013	Ring and surface film	+ + + Opaque	7 - 16	Circular	Smooth, glossy	Low convex	Entire	Mucoid	Coral
3PS014	Pellicle	+ + + Opaque	2 - 9	Irregular	Wrinkled, dull	Raised	Lobate	Tough	Black
3PS015	Thin ring	+ + Opaque	3 - 14	Circular	Smooth, glossy	Low convex	Entire	Mucoid	Coral
3PS016	Ring	+ + Opaque	4 - 18	Irregular	Wrinkled, glossy	Low convex	Lobate	Mucoid	Coral-red
3PS017	Thin ring	+ + Opaque	4 - 10	Circular	Smooth, glossy	Low convex	Lobate	Mucoid	Orange
3PS10'1	Ring	+ + + Opaque	3 - 17	Circular	Smooth, glossy	Flat	Entire	Butyrous	Greyish-white
3PS10'2	Ring	+ + + Opaque	2 - 14	Irregular	Smooth, glossy	Flat	Lobate	Mucoid	Coral-red
3PS10'9	Ring	+ + + Opaque	4 - 11	Irregular	Wrinkled, semi-dull	Slightly raised	Lobate	Mucoid	Creamy
3PM01	Broken pellicle	+ + + Opaque	2 - 15	Circular	Slightly wrinkled, dull	Slightly raised	Entire with a few indentations	Butyrous	Yellowish
3PM02	Thin ring	+ + Opaque	4 - 21	Circular	Smooth, glossy	Low convex	Entire	Mucoid	Pink
3PM03	Thin ring	+ + Opaque	4 - 17	Irregular	Smooth, glossy	Flat	Entire	Mucoid	Pink
3PM04	Thin ring	+ + + Opaque	7 - 24	Irregular	Smooth, glossy	Low convex	Entire	Mucoid	Pink
3PM05	Ring	+ + Opaque	2 - 11	Irregular	Smooth, semi-dull	Low convex	Lobate	Butyrous	Creamy
3PM06	Thin ring	+ + Opaque	3 - 14	Circular	Smooth, glossy	Flat	Entire	Mucoid	Pink
3PM09	Ring	+ + Opaque	3 - 21	Circular	Smooth, glossy	Flat	Entire	Butyrous	Greyish-white

TABLE 5 (CONTINUED)

Isolate	2% Glucose-Yeast Extract-Peptone Water	Density of Growth	Colony Diameter (mm)	Form	Surface	Elevation	Margin	Consistency	Colour
3PM010	Thin ring	+ + Opaque	2 - 14	Irregular	Wrinkled, glossy	Low convex	Lobate	Pasty	Orange
3PM011	Thin ring	+ + + Opaque	3 - 11	Circular	Smooth, glossy	Low convex	Lobate	Mucoid	Orange
3PM012	Thin ring	+ + Opaque	4 - 19	Irregular	Wrinkled, glossy	Low convex	Lobate	Pasty	Orange
3PM013	Thin ring	+ + Opaque	3 - 14	Irregular	Wrinkled, glossy	Low convex	Lobate	Pasty	Orange
3PM014	Thin ring	+ + Opaque	4 - 10	Circular	Smooth, glossy	Flat	Entire	Mucoid	Pink
3PM015	Ring	+ + + Opaque	2 - 13	Irregular	Smooth, glossy	Flat	Lobate	Butyrous	Creamy
3PM016	Thin ring	+ + Opaque	3 - 12	Irregular	Smooth, glossy	Low convex	Lobate	Mucoid	Orange
3PM10'3	Ring	+ + + Opaque	2 - 9	Circular	Smooth, glossy	Slightly raised	Lobate	Mucoid	Creamy
3PB01	Thin ring	+ + + Opaque	4 - 17	Irregular	Wrinkled, glossy	Low convex	Lobate	Mucoid	Orange
3PB02	Ring and surface film	+ + + Opaque	7 - 23	Irregular	Smooth, glossy	Low convex	Entire	Mucoid	Coral
3PB03	Ring	+ + + Opaque	2 - 14	Irregular	Ridges in center, glossy	Umbonate	Lobate	Mucoid	Creamy-brown
3PB04	Ring with surface film	+ + + Opaque	4 - 26	Irregular	Smooth, glossy	Low convex	Entire	Mucoid	Coral
3PB05	Ring	+ + + Opaque	2 - 11	Irregular	Smooth, glossy	Low convex	Entire	Butyrous	Creamy
3PB06	Ring	+ + + Opaque	2 - 17	Irregular	Ridges in center, glossy	Umbonate	Lobate	Mucoid	Creamy-brown
3PB07	Ring	+ + + Opaque	4 - 16	Circular	Smooth, glossy	Flat	Entire	Butyrous	Greyish-white
3PB08	Thin ring	+ + Opaque	7 - 18	Circular	Smooth, glossy	Low convex	Entire	Mucoid	Coral

TABLE 5 (CONTINUED)

Isolate	2% Glucose-Yeast Extract-Peptone Water	Density of Growth	Colony Diameter (mm)	Form	Surface	Elevation	Margin	Consistency	Colour
3PB011	Ring with surface film	+ + + Opaque	6 - 22	Irregular	Smooth, glossy	Low convex	Entire	Mucoid	Coral
3PB012	Pellicle	+ + + Opaque	3 - 11	Irregular	Wrinkled, dull	Raised	Lobate	Tough	Black
3PB013	Thin ring	+ + Opaque	5 - 13	Circular	Smooth, glossy	Slightly raised	Lobate	Mucoid	Orange
3PB014	Thin ring	+ + Opaque	4 - 11	Irregular	Smooth, glossy	Low convex	Lobate	Mucoid	Orange
3PB015	Ring	+ + + Opaque	4 - 14	Irregular	Wrinkled, dull	Slightly raised	Lobate	Viscid	Whitish
3PB016	Ring	+ + + Opaque	2 - 10	Irregular	Wrinkled, semi-dull	Slightly raised	Lobate	Butyrous	Creamy
3PB102	Ring with surface film	+ + + Opaque	3 - 19	Irregular	Smooth, glossy	Low convex	Entire	Mucoid	Coral
4PS01	Broken pellicle	+ + + Opaque	3 - 19	Circular	Slightly wrinkled, dull	Slightly raised	Entire with a few indentations	Butyrous	Yellowish
4PS02	Broken pellicle	+ + + Opaque	5 - 11	Circular	Wrinkled, dull	Slightly raised	Entire with a few indentations	Butyrous	Yellowish
4PS03	Broken pellicle	+ + + Opaque	4 - 17	Circular	Slightly wrinkled, dull	Slightly raised	Entire with a few indentations	Butyrous	Yellowish
4PS05	Ring	+ + Opaque	5 - 27	Irregular	Wrinkled, semi-dull	Slightly raised	Lobate	Pasty	Creamy
4PS07	Ring	+ + Opaque	2 - 11	Circular	Smooth, glossy	Convex	Entire	Butyrous	Red

TABLE 5 (CONTINUED)

Isolate	2% Glucose-Yeast Extract-Peptone Water	Density of Growth	Colony Diameter (mm)	Form	Surface	Elevation	Margin	Consistency	Colour
4PS09	Thin ring	+ + Opaque	2 - 14	Circular	Smooth, glossy	Flat	Entire	Mucoid to slimy	Creamy
4PS010	Broken pellicle	+ + + Opaque	3 - 21	Circular	Smooth, glossy	Slightly raised	Lobate	Butyrous	Creamy
4PS011	Broken pellicle	+ + + Opaque	4 - 17	Circular	Smooth, glossy	Slightly raised	Lobate	Butyrous	Creamy
4PS012	Ring	+ + Opaque	4 - 19	Irregular	Wrinkled, glossy	Low convex	Lobate	Mucoid	Coral-red
4PS013	Thin ring	+ + Opaque	3 - 11	Circular	Smooth, glossy	Low convex	Entire	Mucoid	Pink
4PS014	Ring and surface film	+ + + Opaque	5 - 16	Irregular	Smooth, glossy	Low convex	Entire	Mucoid	Pink
4PM01	Thin ring	+ + + Opaque	3 - 12	Irregular	Wrinkled, glossy	Low convex	Lobate	Pasty	Orange
4PM02	Ring	+ + + Opaque	2 - 11	Irregular	Smooth, glossy	Flat	Lobate	Butyrous	Pink
4PM03	Ring	+ + Opaque	4 - 13	Circular	Smooth, glossy	Slightly raised	Lobate	Butyrous	Creamy
4PM04	Ring	+ + + Opaque	3 - 24	Irregular	Wrinkled, semi-dull	Slightly raised	Lobate	Butyrous	Creamy
4PM05	Thin ring	+ + Opaque	2 - 17	Circular	Smooth, glossy	Flat	Entire	Mucoid to slimy	Creamy
4PM07	Ring	+ + + Opaque	3 - 14	Irregular	Wrinkled, semi-dull	Slightly raised	Lobate	Butyrous	Creamy
4PM010	Thin ring	+ + Opaque	3 - 11	Circular	Smooth, glossy	Flat	Entire	Mucoid	Creamy
4PM011	Ring	+ + + Opaque	2 - 14	Irregular	Wrinkled, glossy	Slightly raised	Lobate	Butyrous	Pink

TABLE 5 (CONTINUED)

Isolate	2% Glucose-Yeast Extract-Peptone Water	Density of Growth	Colony Diameter (mm)	Form	Surface	Elevation	Margin	Consistency	Colour
4PM012	Ring and surface film	+ + + Opaque	7 - 23	Irregular	Wrinkled, glossy	Low convex	Entire	Mucoid	Coral-red
4PM013	Ring and surface film	+ + + Opaque	4 - 17	Irregular	Smooth, glossy	Low convex	Entire	Mucoid	Coral
4PM014	Ring	+ + +	3 - 11	Circular	Smooth, glossy	Low convex	Entire	Butyrous	Tan
4PM015	Thin ring	+ + Opaque	7 - 13	Circular	Smooth, glossy	Low convex	Entire	Mucoid	Pink
4PM016	Thin ring	+ + + Opaque	2 - 16	Circular	Smooth, glossy	Flat	Entire	Mucoid to slimy	Creamy
4PB01	Ring	+ + Opaque	4 - 11	Circular	Smooth, glossy	Flat	Entire	Butyrous	Greyish-white
4PB02	Ring	+ + Opaque	3 - 14	Irregular	Wrinkled, semi-dull	Slightly raised	Lobate	Mucoid	Creamy
4PB04	Broken pellicle	+ + + Opaque	2 - 11	Circular	Wrinkled, glossy	Low convex	Lobate	Mucoid	White
4PB05	Broken pellicle	+ + + Opaque	4 - 7	Circular	Smooth, glossy	Low convex	Entire	Butyrous	Tan
4PB06	Ring	+ + + Opaque	2 - 9	Irregular	Wrinkled, glossy	Slightly raised	Erose	Mucoid	Creamy
4PB011	Ring	+ + + Opaque	3 - 10	Irregular	Wrinkled, hairy	Slightly raised	Lobate	Viscid	Whitish-cream
4PB014	Ring	+ + + Opaque	5 - 16	Circular	Smooth, glossy	Flat	Entire	Butyrous	Greyish-white
4PB015	Ring	+ + Opaque	2 - 11	Irregular	Wrinkled glossy	Slightly raised	Lobate	Mucoid	Creamy

TABLE 5 (CONTINUED)

Isolate	2% Glucose-Yeast Extract-Peptone Water	Density of Growth	Colony Diameter (mm)	Form	Surface	Elevation	Margin	Consistency	Colour
SPS01	Broken pellicle	+ + + Opaque	4 - 21	Irregular	Wrinkled, dull	Slightly raised	Lobate	Pasty	Creamy
SPS02	Ring	+ + + Opaque	2 - 12	Irregular	Ridges in center, glossy	Umbonate	Lobate	Mucoid	Brownish
SPS03	Broken pellicle	+ + + Opaque	7 - 14	Circular	Smooth, glossy	Slightly raised	Lobate	Butyrous	Creamy
SPS04	Broken pellicle	+ + + Opaque	3 - 19	Irregular	Wrinkled, semi-dull	Slightly raised	Lobate	Pasty	Creamy
SPS05	Ring	+ + + Opaque	2 - 11	Circular	Smooth, glossy	Slightly raised	Lobate	Viscid	Whitish-cream
SPS07	Ring	+ + + Opaque	4 - 23	Irregular	Smooth, glossy	Low convex	Lobate	Mucoid	Coral-red
SPS08	Broken pellicle	+ + + Opaque	4 - 17	Irregular	Smooth, semi-dull	Slightly raised	Lobate	Butyrous	Creamy
SPS010	Ring	+ + + Opaque	2 - 10	Irregular	Smooth, glossy	Slightly raised	Lobate	Mucoid	Creamy-brown
SPS011	Thin ring	+ + + Opaque	7 - 20	Irregular	Smooth, glossy	Low convex	Entire	Mucoid	Coral-red
SPM02	Thin ring	+ + Opaque	3 - 14	Circular	Smooth, glossy	Low convex	Entire	Mucoid	Coral
SPM03	Ring and surface film	+ + + Opaque	7 - 11	Circular	Smooth, glossy	Low convex	Entire	Mucoid	Coral
SPM05	Thin ring	+ + Opaque	8 - 24	Irregular	Smooth, glossy	Low convex	Entire	Mucoid	Coral
SPM08	Thin ring	+ + + Opaque	2 - 14	Irregular	Wrinkled, glossy	Low convex	Lobate	Pasty	Orange
SPM015	Ring	+ + Opaque	4 - 16	Circular	Smooth, glossy	Low convex	Lobate	Butyrous	Very Pale Pink

TABLE 5 (CONTINUED)

Isolate	2% Glucose-Yeast Extract-Peptone Water	Density of Growth	Colony Diameter (mm)	Form	Surface	Elevation	Margin	Consistency	Colour
5PB02	Ring	+ + + Opaque	3 - 14	Irregular	Smooth, glossy	Low convex	Lobate	Butyrous	Yellow-tan
5PB011	Broken pellicle	+ + + Opaque	4 - 13	Circular	Slightly wrinkled, dull	Slightly raised	Entire with a few indentations	Butyrous	Greyish-yellow
6PS02	Ring	+ + Opaque	3 - 10	Circular	Smooth, glossy	Low convex	Entire	Butyrous	Creamy
6PS03	Ring	+ + Opaque	3 - 11	Circular	Smooth, glossy	Flat	Entire	Butyrous	Greyish-white
6PS07	Ring	+ + Opaque	2 - 17	Irregular	Wrinkled, glossy	Low convex	Lobate	Mucoid	Coral-red
6PS08	Ring	+ + Opaque	4 - 13	Irregular	Wrinkled, semi-dull	Slightly raised	Lobate	Pasty	Creamy
6PS09	Ring	+ + Opaque	2 - 16	Irregular	Wrinkled, semi-dull	Slightly raised	Lobate	Pasty	Creamy
6PS010	Ring	+ + + Opaque	4 - 17	Circular	Smooth, glossy	Flat	Entire	Butyrous	Greyish-white
6PS011	Ring	+ + Opaque	7 - 16	Circular	Smooth, glossy	Slightly raised	Lobate	Butyrous	Creamy
6PS012	Ring	+ + + Opaque	4 - 12	Circular	Smooth, glossy	Flat	Entire	Butyrous	Greyish-white
6PM01	Broken pellicle	+ + Opaque	3 - 14	Circular	Slightly wrinkled, dull	Slightly raised	Entire with a few indentations	Butyrous	Yellowish
6PM04	Broken pellicle	+ + + Opaque	2 - 11	Circular	Wrinkled, dull	Slightly raised	Entire with a few indentations	Butyrous	Greyish-yellow
6PM06	Ring	+ + Opaque	7 - 10	Circular	Smooth, glossy	Slightly raised	Lobate	Butyrous	Creamy

TABLE 5 (CONTINUED)

Isolate	2% Glucose-Yeast Extract-Peptone Water	Density of Growth	Colony Diameter (mm)	Form	Surface	Elevation	Margin	Consistency	Colour
6PM07	Ring	+ + Opaque	4 - 10	Circular	Smooth, glossy	Slightly raised	Entire	Butyrous	Greyish-white
6PB01	Pellicle	+ + + Opaque	6 - 13	Irregular	Wrinkled, glossy	Slightly raised	Lobate	Membranous	Yellow
6PB02	Ring	+ + + Opaque	3 - 14	Circular	Smooth, glossy	Flat	Entire	Butyrous	Greyish-white
6PB08	Thin ring	+ + Opaque	6 - 11	Circular	Smooth, glossy	Slightly raised	Lobate	Pasty	Orange
6PB015	Ring and surface film	+ + + Opaque	7 - 19	Irregular	Wrinkled, glossy	Low convex	Entire	Mucoid	Coral
7PS01	Pellicle	+ + + Opaque	3 - 10	Circular	Wrinkled, dull	Slightly raised	Lobate	Pasty	Brownish
7PS02	Pellicle	+ + + Opaque	2 - 14	Irregular	Wrinkled, dull	Slightly raised	Lobate	Mucoid	Creamy
7PS03	Ring	+ + Opaque	3 - 14	Irregular	Smooth, semi-dull	Slightly raised	Lobate	Pasty	Creamy
7PS04	Pellicle	+ + + Opaque	2 - 10	Irregular	Wrinkled, hairy	Raised	Lobate	Tough	Black
7PS05	Ring	+ + Opaque	4 - 10	Circular	Smooth, glossy	Flat	Entire	Butyrous	Greyish-white
7PS06	Ring	+ + + Opaque	3 - 14	Circular	Smooth, glossy	Flat	Entire	Butyrous	Greyish-white
7PS08	Broken pellicle	+ + + Opaque	7 - 13	Circular	Wrinkled, semi-dull	Slightly raised	Lobate	Pasty	Creamy
7PS09	Broken pellicle	+ + + Opaque	4 - 23	Irregular	Wrinkled, semi-dull	Slightly raised	Lobate	Pasty	Creamy
7PS010	Pellicle	+ + + Opaque	2 - 11	Irregular	Wrinkled, hairy	Raised	Lobate	Tough	Black

TABLE 5 (CONTINUED)

Isolate	2% Glucose-Yeast Extract-Peptone Water	Density of Growth	Colony Diameter (mm)	Form	Surface	Elevation	Margin	Consistency	Colour
7PS013	Broken pellicle	+ + Opaque	4 - 18	Circular	Slightly wrinkled, dull	Slightly raised	Entire with a few indentations	Butyrous	Yellowish
7PS10'1	Ring	+ + + Opaque	3 - 9	Circular	Smooth, glossy	Flat	Entire	Butyrous	Greyish-yellow
7PS10'2	Ring	+ + Opaque	4 - 15	Circular	Smooth, glossy	Flat	Entire	Butyrous	Greyish-white
7PS10'3	Ring	+ + Opaque	4 - 13	Circular	Smooth, glossy	Flat	Entire	Butyrous	Creamy
7PM01	Ring	+ + + Opaque	6 - 21	Irregular	Wrinkled, dull	Slightly raised	Lobate	Viscid	Brownish
7PM03	Broken pellicle	+ + + Opaque	3 - 12	Circular	Slightly wrinkled, dull	Slightly raised	Entire with a few indentations	Butyrous	Yellowish
7PM05	Thin ring	+ + Opaque	4 - 10	Circular	Smooth, glossy	Flat	Entire	Mucoid	Pink
7PB01	Thin ring	+ + + Opaque	7 - 23	Irregular	Wrinkled, glossy	Low convex	Lobate	Mucoid	Orange
7PB04	Ring	+ + + Opaque	2 - 14	Irregular	Ridges in center, glossy	Umbonate	Lobate	Mucoid	Creamy-brown
7PB07	Ring	+ + + Opaque	3 - 11	Irregular	Wrinkled, glossy	Slightly raised	Erose	Mucoid	Creamy
7PB010	Ring	+ + + Opaque	6 - 13	Irregular	Wrinkled, glossy	Slightly raised	Lobate	Butyrous	Creamy

is characterized by the ridges which appear in the centers of the colonies.

(d) Biochemical characteristics

Biochemical characteristics of the isolates are given in Table 6. These are the data upon which the identifications of the organisms were mainly based. Symbols and methods of evaluating the results are explained in the materials and methods section.

The keying out of the unidentifiable cultures sometimes led to invalid identifications. For example, although culture 1PM05 keyed out to *Oosporidium margaritiferum* on the basis of part of the biochemical data, nevertheless, the remainder of such data did not agree with the physiological characteristics of *O. margaritiferum*. Thus, while the isolate in question assimilated melibiose, D-ribose, L-rhamnose, ribitol, galactitol and citric acid, *O. margaritiferum* assimilated none of these. The isolate produced no spores, whereas distinctive ones are found in cultures of *O. margaritiferum*. Even the colours of the colonies were different; *O. margaritiferum* having a light pink or orange-yellow colour and the isolate showing a creamy colour.

Other unidentifiable species were those containing

TABLE .6

BIOCHEMICAL CHARACTERISTICS

Isolate	Carbon Assimilation Tests																											
	Maltose	i-Erythritol	Cellobiose	Raffinose	Sucrose	D-Mannitol	Melezitose	Salicin	D-Galactose	D-Xylose	Methyl-α-D-Glucoside	Ribitol	Trehalose	L-Arabinose	D-Arabinose	D-Glucitol	Soluble Starch	L-Rhamnose	L-Sorbose	D-Ribose	Melibiose	Lactose	Glycerol	Inulin	Inositol	Ethanol	Galactitol	D-Glucose
1PS01	+	+	+	+	+	+	+	+	+	-	+	+	+	+	-	-	+	+	+	+	+	+	+	-	-	+	-	+
1PS02	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+	-	+	+	+	-	-	+	-	+
1PS03	+	-	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-	-	+	-	+
1PS04	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-	-	+	-	+
1PS10'1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	-	+	+	-	-	+	-	+
1PS10'4	+	+	-	-	+	+	+	-	-	+	-	+	+	-	-	+	-	-	-	+	-	-	+	-	-	+	-	+
1PS10'7	+	-	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-	+	+	+	-	-	+	-	-	+	-	+
1PS10'9	+	+	-	-	+	+	+	-	-	-	-	+	+	-	-	+	-	-	-	-	-	-	-	-	-	+	-	+
1PD01	+	-	-	+	+	+	+	+	-	+	+	+	+	+	-	+	-	-	+	+	-	-	+	-	-	+	-	+
1PD02	+	+	-	-	+	+	+	-	-	-	-	+	-	+	-	+	-	-	+	+	-	-	+	-	-	+	-	+
1PD03	+	-	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-	+	+	-	-	-	+	-	-	+	-	+
1PD04	+	-	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-	-	+	+	-	-	+	-	-	+	-	+
1PD05	-	-	-	-	+	-	+	[illegible]	-	-	-	+	+	-	-	-	+	+	+	+	+	-	-	-	-	+	+	+
1PD06	+	-	+	+	+	+	+	+	-	+	+	+	+	+	-	+	-	-	+	+	-	-	+	-	-	+	-	+
1PD07	+	+	-	-	+	+	+	-	-	-	-	-	+	-	-	+	-	-	-	+	-	-	+	-	-	+	-	+
1PD08	+	-	-	-	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+	-	-	-	+	-	-	+	-	+
1PD09	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-	-	+	-	+
1PD011	+	+	+	-	+	+	-	+	+	+	+	+	+	+	+	+	-	+	+	-	-	-	+	-	-	+	-	+
1PD013	+	+	-	-	+	+	+	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-	+	-	-	+	-	+
1PB06	+	-	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-	+	+	-	-	-	+	-	-	+	-	+
1PB07	+	-	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-	-	+	-	+
1PB09	+	-	-	+	+	+	+	+	-	+	+	+	+	+	+	-	-	-	+	+	-	-	+	-	-	+	-	+
1PB010	+	-	-	+	+	+	+	+	-	+	+	+	+	+	+	-	-	-	+	+	-	-	+	-	-	+	-	+
2PS01	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-	-	+	-	+
2PS02	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+	-	-	-	-	-	+	+	+
2PS03	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-	-	+	-	+

Isolate	Carbon Assimilation Tests (cont.)									Fermentation Tests																
	Citric Acid	Succinic Acid	DL-Lactic Acid	2-Keto-Gluconic Acid	Glucono-δ-Lactone	Potassium Nitrate Assimilation	Ethylamine HCl Assimilation	Riboflavin Production	Starch Production	Maltose	D-Glucose	Lactose	Raffinose	Sucrose	Cellobiose	D-Galactose	Melibiose	Trehalose	Melezitose	Inulin	Vitamin-Free Medium Growth	37C Growth	Cycloheximide Resistance	50% Glucose Growth	Arbutin Growth	Gelatin Liquefaction
1PS01	+	+	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	w
1PS02	+	+	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-
1PS03	+	+	+	-	-	-	-	+	-	-	+	-	-	-	-	+	-	-	-	-	-	-	+	+	+	-
1PS04	+	+	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-
1PS10'1	+	+	+	-	-	-	-	+	-	-	+	-	-	-	-	+	-	-	-	-	-	-	+	+	+	-
1PS10'4	+	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
1PS10'7	+	+	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-
1PS10'9	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
1PD01	+	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-
1PD02	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1PD03	+	+	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
1PD04	+	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-
1PD05	+	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
1PD06	+	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-
1PD07	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
1PD08	+	-	+	-	-	-	-	-	-	+	+	-	-	+	-	+	-	+	+	-	-	+	-	-	-	-
1PD09	+	+	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-
1PD011	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-	+	-	-	+	+	-
1PD013	-	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-
1PB06	+	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-
1PB07	+	+	+	-	-	-	-	+	-	-	+	-	-	-	-	+	-	-	-	-	-	-	+	+	+	-
1PB09	+	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-
1PB010	+	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	+	-
2PS01	+	+	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-
2PS02	+	+	+	-	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2PS03	+	+	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-

TABLE 6 (CONTINUED)

Isolate	Carbon Assimilation Tests																																					Fermentation Tests																	
	Maltose	i-Erythritol	Cellobiose	Raffinose	Sucrose	D-Mannitol	Melezitose	Salicin	D-Galactose	D-Xylose	Methyl-α-D-Glucoside	Ribitol	Trehalose	L-Arabinose	D-Arabinose	D-Glucitol	Soluble Starch	L-Rhamnose	L-Sorbose	D-Ribose	Melibiose	Lactose	Glycerol	Inulin	Inositol	Ethanol	Galactitol	D-Glucose	Citric Acid	Succinic Acid	DL-Lactic Acid	2-Keto-Gluconic Acid	Glucono-δ-Lactone	Potassium Nitrate Assimilation	Ethylamine HCl Assimilation	Riboflavin Production	Starch Production	Maltose	D-Glucose	Lactose	Raffinose	Sucrose	Cellobiose	D-Galactose	Melibiose	Trehalose	Melezitose	Inulin	Vitamin-free Medium Growth	37C Growth	Cycloheximide Resistance	50% Glucose Growth	Arbutin Growth	Gelatin Liquefaction
2PS04	+	-	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-	-	+	-	+	+	+	+	-	-	-	-	+	-	+	+	-	-	-	-	+	-	-	-	-	-	-	+	+	+	-
2PS05	+	-	+	-	+	+	+	-	+	+	+	+	+	+	-	+	+	-	+	-	-	-	+	-	-	+	-	-	+	+	+	-	-	-	-	-	-	+	+	-	-	+	-	+	-	+	+	-	-	+	-	-	-	-
2PS06	+	-	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-	-	+	-	+	+	+	+	-	-	-	-	+	-	+	+	-	-	-	-	+	-	-	-	-	-	-	+	+	+	-
2PS07	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-	-	+	-	+	+	+	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-
2PS08	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	-	+	-	-	+	-	+	+	+	+	-	-	-	-	+	-	-	+	-	-	-	-	+	-	-	-	-	-	-	+	+	+	-
2PS09	+	-	-	+	+	+	+	+	-	+	+	+	+	+	+	+	-	+	+	+	-	-	+	-	-	+	-	+	+	+	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-
2PS010	+	-	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-	-	-	-	+	-	+	-	+	+	+	+	+	+	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2PS011	+	-	-	+	+	+	+	+	-	+	+	+	+	+	+	+	-	+	+	+	-	-	+	-	-	+	-	+	+	+	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-
2PS012	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-	-	+	-	+	+	+	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-
2PS013	+	+	+	-	+	+	-	+	+	+	+	+	+	+	+	+	-	+	+	+	-	-	+	-	-	+	-	+	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-	+	-	-	+	+	-
2PS014	+	-	-	-	+	+	+	-	+	+	+	+	+	+	-	+	+	-	+	-	-	-	+	-	-	+	-	+	-	+	+	-	-	-	-	-	-	+	+	-	-	+	-	+	-	+	+	-	-	+	-	-	-	-
2PS015	+	+	+	-	+	+	-	+	+	+	+	+	+	+	+	+	-	+	-	+	-	-	+	-	-	+	-	+	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-	+	-	-	+	+	-
2PS016	-	-	+	+	+	+	-	+	+	+	-	+	+	+	+	+	-	+	+	+	-	-	+	-	-	+	+	+	-	+	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
2PS017	+	-	-	-	+	+	+	+	+	+	+	+	+	-	-	+	+	-	+	-	-	-	[illegible]	-	-	+	-	+	+	+	+	-	-	-	-	-	-	+	+	-	-	+	-	+	-	+	+	-	-	+	-	-	-	-
2PS10'2	+	-	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-	-	-	-	+	-	-	-	+	+	+	+	+	+	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2PS10'3	+	+	+	-	+	+	-	+	+	+	+	+	+	+	+	+	-	+	[illegible]	+	-	-	+	-	-	+	-	+	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	+	+	-
2PM01	+	+	-	-	+	+	+	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
2PM02	+	+	+	-	+	+	-	+	+	+	+	+	+	+	+	+	-	+	+	+	-	-	+	-	-	+	-	+	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-	+	-	-	+	+	-
2PM03	+	+	+	-	+	+	-	+	+	+	+	+	+	+	+	+	-	+	-	+	-	-	+	-	-	+	-	+	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-	+	-	-	+	+	-
2PM04	+	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-	-	+	+	-	-	+	-	-	+	-	+	+	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-
2PM05	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	-	-	+	-	-	+	-	+	+	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-
2PM06	+	-	-	+	+	+	+	+	-	+	+	+	+	+	+	-	-	-	+	+	-	-	+	-	-	+	-	+	+	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-
2PM07	+	-	-	+	+	+	+	+	-	+	+	+	+	+	+	-	-	-	+	+	-	-	+	-	-	+	-	+	[illegible]	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-
2PM08	+	-	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-	+	+	+	-	-	+	-	-	+	-	+	+	+	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-
2PM011	+	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-	-	+	+	-	-	+	-	-	+	-	+	+	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-
2PM012	+	-	-	+	+	+	+	+	-	+	+	+	+	+	+	+	-	-	+	+	-	-	+	-	-	+	-	+	+	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-

TABLE 6 (CONTINUED)

Isolate	Carbon Assimilation Tests																																
	Maltose	i-Erythritol	Cellobiose	Raffinose	Sucrose	D-Mannitol	Melezitose	Salicin	D-Galactose	D-Xylose	Methyl-α-D-Glucoside	Ribitol	Trehalose	L-Arabinose	D-Arabinose	D-Glucitol	Soluble Starch	L-Rhamnose	L-Sorbose	D-Ribose	Melibiose	Lactose	Glycerol	Inulin	Inositol	Ethanol	Galactitol	D-Glucose	Citric Acid	Succinic Acid	DL-Lactic Acid	2-Keto-Gluconic Acid	Glucono-δ-Lactone
2PMD13	+	+	+	-	+	+	-	+	+	+	+	+	+	+	+	+	-	+	+	+	-	-	+	-	-	+	-	+	-	+	-	-	-
2PMD14	+	+	+	-	+	+	-	+	+	+	+	+	+	+	+	+	-	+	-	+	-	-	+	-	-	+	-	+	-	+	-	-	-
2PMD15	+	-	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-	-	+	+	-	-	+	-	-	+	-	+	+	+	+	-	+
2PMD16	+	+	+	-	+	+	-	+	+	+	+	+	+	+	+	+	-	+	+	+	-	-	+	-	-	+	-	+	-	+	-	-	-
2PM10'1	+	-	-	+	+	+	+	+	-	+	+	+	+	+	+	-	-	-	+	+	-	-	+	-	-	-	-	+	+	+	+	-	+
2PM10'2	-	-	-	-	+	-	+	-	-	-	-	+	+	-	-	-	+	+	+	+	+	-	-	-	-	+	+	+	-	-	-	-	-
2PB02	+	-	+	+	+	+	+	+	-	+	+	-	+	-	-	+	+	+	-	-	-	-	+	-	-	+	-	+	+	+	+	-	-
2PB04	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-	-	+	-	+	+	+	+	-	-
2PB05	+	-	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-	+	+	+	-	-	+	-	-	+	-	+	+	+	-	+	+
2PS05	+	-	-	-	-	-	+	-	-	-	-	+	+	-	-	-	+	+	+	+	+	-	-	-	-	+	+	+	-	-	-	-	-
2PB07	-	-	-	+	+	+	-	+	+	+	-	+	+	-	+	+	-	+	+	+	+	-	+	-	-	+	-	+	-	+	-	+	+
2PB08	-	-	-	-	+	+	-	-	-	-	-	+	+	-	-	-	+	+	+	+	+	-	-	-	-	+	+	+	-	-	-	-	-
2PB09	+	-	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-	-	+	-	+	+	+	+	-	-
2PB010	+	-	-	-	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+	-	-	-	+	-	-	+	-	+	+	+	+	-	-
2PB012	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-	-	+	-	+	+	+	+	-	-
2PB013	+	-	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-	-	+	-	+	+	+	+	-	-
2PB014	+	-	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-	-	+	-	+	+	+	+	-	-
2PB10'1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	-	-	+	-	+	-	+	+	-	-
2PB10'2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+	+	+	+	+	-	-
2PB10'11	+	-	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-	-	+	-	+	+	+	+	-	-
2PB10'12	+	-	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	-	-	+	-	+	-	+	+	-	-
2PB10'13	+	+	+	-	+	+	+	+	+	+	+	+	+	+	±	+	+	+	+	+	+	±	[illegible]	-	-	-	+	+	+	-	+	-	-
3PS01	+	-	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-	±	+	+	-	-	+	-	-	+	-	+	+	+	+	-	+
3PS02	+	-	+	+	+	+	+	-	-	+	+	-	+	-	-	+	+	+	-	-	-	-	+	-	-	+	-	+	+	+	+	-	-
3PS03	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	-	-	+	-	+	+	+	+	-	±
3PS04	+	-	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	-	+	-	+	-	-	+	-	+	+	+	+	-	-

Isolate	Potassium Nitrate Assimilation	Ethylamine HCl Assimilation	Riboflavin Production	Starch Production	Fermentation Tests											Vitamin-Free Medium Growth	37C Growth	Cycloheximide Resistance	50% Glucose Growth	Arbutin Growth	Gelatin Liquefaction
					Maltose	D-Glucose	Lactose	Raffinose	Sucrose	Cellobiose	D-Galactose	Melibiose	Trehalose	Melezitose	Inulin						
2PMD13	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-	+	-	-	+	+	-
2PMD14	-	-	-	-	-	+	-	-	-	-	+	-	+	-	-	+	-	-	+	+	-
2PMD15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-
2PMD16	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-	+	-	-	+	+	-
2PM10'1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-
2PM10'2	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
2PB02	-	-	-	-	-	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
2PB04	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-
2PB05	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-
2PS05	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
2PB07	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
2PB08	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-
2PB09	-	-	+	-	-	+	-	-	-	-	+	-	-	-	-	-	-	+	+	+	-
2PB010	-	-	-	-	+	+	-	-	+	-	+	-	+	+	-	-	+	-	-	-	-
2PB012	-	-	+	-	+	+	-	-	-	-	+	-	-	-	-	-	-	+	+	+	-
2PB013	-	-	+	-	-	+	-	-	-	-	+	-	-	-	-	-	-	+	+	+	-
2PB014	-	-	+	-	+	+	-	-	-	-	+	-	-	-	-	-	-	+	+	+	-
2PB10'1	-	-	+	-	-	+	-	-	-	-	+	-	-	-	-	-	-	+	+	+	-
2PB10'2	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-
2PB10'11	-	-	+	-	+	+	-	-	-	-	+	-	-	-	-	-	-	+	+	+	-
2PB10'12	-	-	+	-	-	+	-	-	-	-	+	-	-	-	-	-	-	+	+	+	-
2PB10'13	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-
3PS01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-
3PS02	-	-	-	-	-	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
3PS03	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-
3PS04	±	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-

TABLE 6 (CONTINUED)

Carbon Assimilation Tests

Isolate	Maltose	i-Erythritol	Cellobiose	Raffinose	Sucrose	D-Mannitol	Melezitose	Salicin	D-Galactose	D-Xylose	Methyl-α-D-Glucoside	Ribitol	Trehalose	L-Arabinose	D-Arabinose	D-Glucitol	Soluble Starch	L-Rhamnose	L-Sorbose	D-Ribose	Melibiose	Lactose	Glycerol	Inulin	Inositol	Ethanol	Galactitol	D-Glucose	Citric Acid	Succinic Acid	DL-Lactic Acid	2-Keto-Gluconic Acid	Glucono-δ-Lactone
3PS05	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	-	-	+	-	+	+	+	+	-	-
3PS06	+	-	-	-	+	+	+	-	+	+	+	+	+	+	-	+	+	-	+	-	-	-	-	-	-	+	-	+	+	+	+	-	-
3PS07	+	-	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	-	+	-	+	-	-	+	-	+	+	+	+	-	-
3PS08	+	-	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	-	-	+	-	+	-	+	+	-	-
3PS09	+	-	-	+	+	+	+	+	-	+	+	+	+	+	+	-	-	-	+	+	-	-	+	-	-	-	-	+	+	+	+	-	+
3PS010	+	-	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-	+	+	+	-	+	-	-	-	+	+	+	+	+	+	+	+
3PS011	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	-	+	-	+	-	-	+	-	+	+	+	+	-	-
3PS012	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	-	+	+	+	-	-	+	-	+	+	+	+	-	-
3PS013	+	-	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-	-	+	+	-	-	±	-	-	+	-	+	+	+	+	-	+
3PS014	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-	+	-	-	-	+	±	+	+	+	+	+	+
3PS015	+	-	-	+	+	+	+	+	-	+	+	+	+	+	+	-	-	-	+	+	-	-	+	-	-	-	-	+	+	+	+	-	+
3PS016	-	±	+	+	+	+	-	+	+	±	-	+	+	+	+	+	-	+	+	+	-	-	+	-	-	+	+	+	-	+	-	+	+
3PS017	+	-	-	+	+	+	+	+	-	+	+	+	+	+	+	+	-	+	+	+	-	-	+	-	-	+	-	+	+	+	-	+	+
3PS10'1	+	-	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-	-	+	-	+	+	+	+	-	-
3PS10'2	-	-	-	+	+	+	-	+	+	+	-	+	+	+	+	+	[illegible]	+	+	+	-	-	+	-	-	+	+	+	-	+	-	+	+
3PS10'9	-	-	-	-	+	+	+	±	+	-	-	±	+	-	-	-	+	+	+	+	+	-	-	-	-	+	+	+	+	+	+	±	±
3PM01	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	-	+	+	+	-	-	+	-	+	+	+	+	-	-
3PM02	+	-	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-	-	+	+	-	-	+	-	±	+	-	+	+	+	+	-	+
3PM03	+	-	-	+	+	+	+	+	-	+	+	+	+	+	+	-	-	-	+	+	-	-	+	-	-	±	-	+	+	+	+	-	+
3PM04	+	-	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-	-	+	+	-	-	+	-	-	+	-	+	+	+	+	-	+
3PM05	-	-	-	-	+	-	+	±	-	-	-	+	+	-	-	-	+	+	-	-	-	+	+	+	+	+	-	+	-	-	+	+	+
3PM06	+	-	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-	-	+	+	-	-	+	-	-	±	-	+	+	+	+	-	+
3PM09	+	-	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-	-	+	-	+	+	+	+	-	-
3PM010	+	-	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-	+	+	+	-	-	+	-	-	+	-	+	+	+	-	+	+
3PM011	+	-	-	+	+	+	+	+	-	+	+	+	+	+	+	+	-	+	+	+	-	-	+	-	-	+	-	+	+	+	-	+	+
3PM012	+	-	-	+	+	+	+	+	-	+	+	+	+	+	+	+	-	-	+	+	-	-	+	-	-	+	-	+	-	+	-	+	+

Other Tests and Fermentation Tests

Isolate	Potassium Nitrate Assimilation	Ethylamine HCl Assimilation	Riboflavin Production	Starch Production	Fermentation: Maltose	Fermentation: D-Glucose	Fermentation: Lactose	Fermentation: Raffinose	Fermentation: Sucrose	Fermentation: Cellobiose	Fermentation: D-Galactose	Fermentation: Melibiose	Fermentation: Trehalose	Fermentation: Melezitose	Fermentation: Inulin	Vitamin-Free Medium Growth	37C Growth	Cycloheximide Resistance	50% Glucose Growth	Arbutin Growth	Gelatin Liquefaction
3PS05	-	-	+	-	±	+	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-
3PS06	-	-	-	-	+	+	-	-	+	-	+	-	+	+	-	-	+	-	-	-	-
3PS07	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-
3PS08	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-
3PS09	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	+	-	-	-
3PS010	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3PS011	-	-	+	-	±	+	-	-	-	-	+	-	-	-	-	-	-	+	+	+	-
3PS012	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-
3PS013	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-
3PS014	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3PS015	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-
3PS016	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
3PS017	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-
3PS10'1	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-
3PS10'2	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
3PS10'9	-	-	-	-	-	+	-	-	-	±	+	±	-	-	-	-	+	-	-	-	-
3PM01	-	-	+	-	±	+	-	-	-	-	±	-	-	-	-	-	-	+	+	+	-
3PM02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-
3PM03	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-
3PM04	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-
3PM05	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
3PM06	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-
3PM09	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-
3PM010	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-
3PM011	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-
3PM012	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-

TABLE 6 (CONTINUED)

Isolate	Carbon Assimilation Tests																																					Fermentation Tests																
	Maltose	L-Erythritol	Cellobiose	Raffinose	Sucrose	D-Mannitol	Melezitose	Salicin	D-Galactose	D-Xylose	Methyl-α-D-Glucoside	Ribitol	Trehalose	L-Arabinose	D-Arabinose	D-Glucitol	Soluble Starch	L-Rhamnose	L-Sorbose	D-Ribose	Melibiose	Lactose	Glycerol	Inulin	Inositol	Ethanol	Galactitol	D-Glucose	Citric Acid	Succinic Acid	DL-Lactic Acid	2-Keto-Gluconic Acid	Glucono-δ-Lactone	Potassium Nitrate Assimilation	Ethylamine HCl Assimilation	Riboflavin Production	Starch Production	Maltose	D-Glucose	Lactose	Raffinose	Sucrose	Cellobiose	D-Galactose	Melibiose	Trehalose	Melezitose	Inulin	Vitamin-Free Medium Growth	37C Growth	Cycloheximide Resistance	50% Glucose Growth	Arbutin Growth	Gelatin Liquefaction
3PM013	+	-	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-	+	+	+	-	-	+	-	-	+	-	+	+	+	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-
3PM014	+	-	+	+	+	+	+	-	-	+	+	+	+	+	+	+	-	-	+	+	-	-	+	-	-	+	-	+	+	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-
3PM015	±	±	±	±	±	±	+	±	±	±	-	±	±	+	+	±	+	+	±	+	+	±	±	±	±	+	+	+	±	±	+	±	-	+	-	-	-	-	+	-	-	±	-	±	±	-	-	-	-	+	-	-	-	-
3PM016	+	-	-	+	+	+	+	±	-	±	-	+	+	+	+	+	-	-	+	+	-	-	+	-	-	+	-	+	+	+	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-
3PM10'3	-	-	-	-	+	+	+	+	+	+	+	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3PB01	+	-	+	+	+	+	+	+	±	+	+	+	+	+	+	+	-	+	+	+	-	-	+	-	-	+	-	+	+	+	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-
3PB02	+	-	-	+	+	+	+	+	-	+	+	+	+	+	+	-	-	-	+	+	-	-	+	-	-	+	-	+	+	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-
3PB03	+	+	-	-	+	+	+	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	±	-
3PB04	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	+	+	-	-	+	-	-	+	-	+	+	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
3PB05	+	-	-	-	-	-	+	±	±	-	±	-	±	+	+	-	+	+	+	+	+	±	±	-	-	+	+	+	+	+	±	±	-	-	±	-	-	-	+	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-
3PB06	+	+	-	-	+	+	+	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	+	±	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
3PB07	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	-	+	+	+	-	-	+	-	+	+	+	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-
3PB08	+	-	-	+	+	±	+	+	+	+	+	+	+	+	+	-	-	-	+	+	-	-	+	-	-	+	-	+	+	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-
3PB011	+	-	+	+	+	±	+	+	-	+	+	+	+	+	+	+	-	-	+	+	-	-	+	-	-	+	-	+	+	+	+	-	+	-	±	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-
3PB012	+	-	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+	±	±	-	+	-	-	-	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3PB013	+	-	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-	+	+	+	-	-	+	-	-	+	-	+	+	+	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-
3PB014	+	-	+	+	+	+	+	±	-	+	+	+	+	+	+	+	-	+	-	+	±	-	+	-	-	+	-	+	+	+	±	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-
3PB015	+	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	-	-	+	-	-	+	-	+	+	+	+	-	-	-	-	-	-	+	+	-	-	+	-	+	-	+	+	-	-	+	-	-	-	-
3PB016	±	-	-	-	+	-	+	±	+	+	+	-	-	+	+	+	+	+	+	-	-	-	-	-	+	+	-	+	-	-	-	-	-	-	-	-	-	±	+	±	±	-	-	-	-	-	-	-	-	+	-	-	-	+
3PB10'2	+	-	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-	-	+	+	-	-	+	-	-	+	-	+	+	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-
4PS01	+	-	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-	-	+	-	+	+	+	+	-	-	-	-	+	-	-	±	-	-	±	-	-	-	-	-	-	-	-	+	+	+	-
4PS02	+	+	+	+	+	+	+	+	+	+	+	±	+	+	-	+	+	+	+	+	+	+	+	-	-	+	-	+	+	+	+	-	-	-	-	+	-	-	-	-	-	-	-	±	-	-	-	-	-	-	+	+	+	-
4PS03	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	-	+	-	-	+	-	+	+	+	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-
4PS05	+	+	+	-	+	+	-	+	+	+	+	+	+	+	+	+	-	+	+	+	-	-	+	-	-	+	-	+	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-	+	-	-	+	+	-
4PS07	+	-	+	+	+	+	+	+	+	±	-	+	+	+	+	+	+	+	-	-	-	-	+	-	+	-	+	+	+	+	+	+	+	+	±	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4PS09	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	±	±	+	-	+	-	-	+	+	+	+	+	+	+	+	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

TABLE 6 (CONTINUED)

Isolate	Carbon Assimilation Tests																																					Fermentation Tests																	
	Maltose	L-Erythritol	Cellobiose	Raffinose	Sucrose	D-Mannitol	Melezitose	Salicin	D-Galactose	D-Xylose	Methyl-α-D-Glucoside	Ribitol	Trehalose	L-Arabinose	D-Arabinose	D-Glucitol	Soluble Starch	L-Rhamnose	L-Sorbose	D-Ribose	Melibiose	Lactose	Glycerol	Inulin	Inositol	Ethanol	Galactitol	D-Glucose	Citric Acid	Succinic Acid	DL-Lactic Acid	2-Keto-Gluconic Acid	Glucono-δ-Lactone	Potassium Nitrate Assimilation	Ethylamine HCl Assimilation	Riboflavin Production	Starch Production	Maltose	D-Glucose	Lactose	Raffinose	Sucrose	Cellobiose	D-Galactose	Melibiose	Trehalose	Melezitose	Inulin	Vitamin-free Medium Growth	37C Growth	Cycloheximide Resistance	50% Glucose Growth	Arbutin Growth	Gelatin Liquefaction
4PS010	+	+	+	-	+	+	-	+	+	+	+	+	+	+	+	+	-	+	-	+	-	-	+	-	-	+	-	+	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-	+	-	-	+	+	-
4PS011	+	+	+	-	+	+	-	+	+	+	+	+	+	+	+	+	-	+	-	+	-	-	+	-	-	+	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	+	-
4PS012	-	-	-	+	+	+	-	+	+	+	-	+	+	+	+	+	-	+	+	+	-	-	+	-	-	+	+	+	-	+	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
4PS013	+	-	-	+	+	+	+	+	-	+	+	+	+	+	+	-	-	-	+	+	-	-	+	-	-	+	-	+	+	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-
4PS014	+	-	-	+	+	±	+	+	+	+	+	+	+	+	+	-	-	-	+	+	±	-	+	-	-	+	-	+	+	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-
4PM01	+	-	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-	+	+	+	-	-	+	-	-	+	-	+	+	+	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-
4PM02	+	-	-	-	-	-	+	+	-	-	+	+	+	+	+	+	±	+	+	+	+	-	-	-	-	-	-	+	+	+	+	+	+	+	-	-	-	+	+	-	±	±	-	-	-	-	-	-	-	-	-	-	-	-
4PM03	+	+	+	-	+	+	±	+	+	+	+	+	+	+	+	+	-	+	+	+	-	-	+	-	-	+	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	+	-
4PM04	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-	-	+	-	-	+	-	+	±	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	+	-
4PM05	+	-	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+	±	+	-	+	-	-	+	+	+	+	+	+	+	+	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4PM07	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	±	±	+	+	+	+	+	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
4PM010	+	-	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	-	-	+	-	+	-	-	+	+	±	+	+	+	+	+	+	+	-	-	[illegible]	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4PM011	+	-	-	-	-	-	+	+	+	±	+	+	+	+	±	+	-	-	-	-	-	-	±	+	+	+	+	+	+	-	+	+	+	±	±	-	-	-	+	-	±	±	±	-	±	±	-	-	-	-	-	-	-	-
4PM012	+	-	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-	-	+	+	-	-	+	-	-	+	-	+	+	+	+	-	±	-	±	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-
4PM013	+	-	+	+	+	±	+	+	±	+	+	+	+	+	+	+	-	-	+	+	-	-	+	-	-	+	-	+	+	+	+	-	+	-	-	-	-	-	-	-	-	±	-	-	-	-	-	-	-	+	+	-	-	-
4PM014	±	±	±	±	±	±	±	±	±	+	±	±	±	±	±	±	-	±	-	-	+	±	+	-	-	+	±	+	±	±	±	±	+	-	-	-	-	-	±	-	-	±	±	-	-	-	-	-	-	-	-	-	-	-
4PM015	+	-	-	+	+	+	+	+	-	+	+	+	+	+	+	-	-	-	+	+	-	-	+	-	-	+	-	+	+	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-
4PM016	+	-	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+	±	+	-	+	-	-	+	+	+	+	+	+	+	+	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4PB01	+	-	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	-	+	-	-	+	-	+	+	+	+	-	-	-	-	+	-	-	±	-	-	±	-	-	-	-	-	-	-	-	+	+	+	-
4PB02	-	-	-	-	+	-	+	+	+	+	+	+	+	+	+	+	-	±	±	-	-	-	-	-	-	+	-	+	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4PB04	+	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	-	-	+	-	-	-	-	+	+	+	+	-	-	-	-	+	-	-	±	-	±	±	±	±	-	-	-	-	-	-	-	-	-	-
4PB05	-	-	-	-	+	-	±	±	+	+	+	+	+	-	-	±	-	+	+	+	+	-	+	+	+	-	-	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
4PB06	+	-	+	+	+	+	+	+	+	+	+	-	+	-	-	+	-	+	+	±	-	+	+	-	-	+	+	+	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	+
4PB011	+	-	-	-	+	+	+	±	+	+	+	+	+	+	-	+	+	-	-	-	-	-	+	-	-	+	-	+	+	+	+	-	-	-	-	-	-	+	+	-	-	+	-	+	-	+	+	-	-	+	-	-	-	-
4PB014	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	-	+	-	-	+	-	+	+	+	+	-	-	-	-	+	-	±	±	-	-	±	-	±	-	-	-	-	-	-	+	+	+	-
4PB015	±	±	±	±	+	±	±	±	±	-	±	±	±	+	±	±	±	±	±	±	+	±	±	+	±	-	±	+	±	±	±	-	±	±	-	-	-	-	+	-	-	-	-	-	-	±	±	-	-	+	-	-	-	-

TABLE 6 (CONTINUED)

Isolate	Carbon Assimilation Tests																																					Fermentation Tests																
	Maltose	L-Erythritol	Cellobiose	Raffinose	Sucrose	D-Mannitol	Melezitose	Salicin	D-Galactose	D-Xylose	Methyl-α-D-Glucoside	Ribitol	Trehalose	L-Arabinose	D-Arabinose	D-Glucitol	Soluble Starch	L-Rhamnose	L-Sorbose	D-Ribose	Melibiose	Lactose	Glycerol	Inulin	Inositol	Ethanol	Galactitol	D-Glucose	Citric Acid	Succinic Acid	DL-Lactic Acid	2-Keto-Gluconic Acid	Glucono-δ-Lactone	Potassium Nitrate Assimilation	Ethylamine HCl Assimilation	Riboflavin Production	Starch Production	Maltose	D-Glucose	Lactose	Raffinose	Sucrose	Cellobiose	D-Galactose	Melibiose	Trehalose	Melezitose	Inulin	Vitamin-free Medium Test	37C Growth Test	Cycloheximide Resistance	50% Glucose Growth	Arbutin Growth	Gelatin Liquefaction
5PS01	+	+	+	-	+	+	-	+	+	+	+	+	+	+	+	+	-	+	+	+	-	-	+	-	-	+	-	+	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-	+	-	-	+	+	-
5PS02	+	+	-	-	+	+	+	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-	±	-	-	+	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
5PS03	+	+	+	-	+	+	-	+	+	+	+	+	+	+	+	+	-	+	±	+	-	-	+	-	-	+	-	+	-	+	-	-	-	-	-	-	-	-	±	-	-	-	-	±	-	-	-	-	+	-	-	+	+	-
5PS04	+	+	+	-	+	+	-	+	+	+	+	+	+	+	+	+	-	+	±	+	-	-	+	-	-	+	-	+	-	+	-	-	-	-	-	-	-	-	±	-	-	-	-	±	-	-	-	-	+	-	-	+	+	-
5PS05	+	-	-	-	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+	-	-	-	+	-	-	+	-	+	+	+	+	-	-	-	-	-	-	+	+	-	-	+	-	+	-	+	+	-	-	+	-	-	-	-
5PS07	-	-	+	+	+	+	-	+	+	+	-	+	+	+	+	+	-	+	+	+	-	-	+	-	-	+	+	+	-	+	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
5PS08	+	+	+	-	+	+	-	+	+	+	+	+	+	-	+	+	-	+	+	+	-	-	+	-	-	+	-	+	-	+	-	-	-	-	-	-	-	-	±	-	-	-	-	-	-	-	-	-	+	-	-	+	+	-
5PS010	+	+	-	-	+	+	+	-	-	-	-	±	+	-	-	+	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
5PS011	+	-	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-	-	+	+	-	-	+	-	-	+	-	+	+	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-
5PM02	+	-	+	+	+	+	+	-	+	+	+	+	+	+	+	±	-	-	+	+	-	-	+	-	-	+	-	+	+	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-
5PM03	+	-	+	+	±	±	+	+	-	+	+	+	+	+	+	+	-	-	+	+	-	-	+	-	-	+	-	+	+	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-
5PM05	+	-	+	+	+	±	+	+	-	+	+	+	+	+	+	±	-	-	+	+	-	-	+	-	-	+	-	+	+	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-
5PM08	+	-	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-	+	+	+	-	-	+	-	-	+	-	+	+	+	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-
5PM15	-	-	+	-	-	+	-	+	+	+	-	-	+	-	+	+	-	-	+	-	-	-	+	-	-	+	-	+	±	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5PB02	+	-	-	-	-	+	-	-	-	-	+	+	-	-	-	+	+	+	+	+	+	-	-	-	-	+	+	+	+	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
5PB011	+	+	+	+	+	+	+	+	+	+	+	±	+	+	-	+	+	+	+	+	+	+	+	-	-	+	-	+	+	+	+	-	±	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-
6PS02	±	±	±	-	-	±	±	-	+	+	+	+	±	±	±	±	±	±	+	+	±	±	±	-	-	±	-	+	±	±	±	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6PS03	+	-	+	+	+	+	+	+	+	+	+	±	+	+	-	+	+	+	+	+	+	+	+	-	-	+	-	+	+	+	+	-	-	-	-	+	-	+	+	-	-	-	-	±	-	-	-	-	-	-	+	+	+	-
6PS07	-	-	-	+	+	+	-	+	+	+	-	+	+	+	+	+	-	+	+	+	-	-	+	-	-	+	+	+	-	+	-	+	+	+	-	-	-	-	±	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
6PS08	+	+	+	-	+	+	-	+	+	+	+	+	+	+	+	+	-	+	+	+	-	-	+	-	-	+	-	+	±	+	-	-	-	-	-	-	-	-	±	-	-	-	-	-	-	-	-	-	+	-	-	+	+	-
6PS09	+	+	+	-	+	+	-	+	+	+	+	+	+	+	+	+	-	+	±	+	-	-	+	-	-	+	-	+	-	+	-	-	-	-	-	-	-	-	±	-	-	-	-	-	-	-	-	-	+	-	-	+	+	-
6PS010	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	-	-	+	-	+	+	+	±	-	-	-	-	+	-	-	+	-	-	-	-	±	-	-	-	-	-	-	+	+	+	-
6PS011	+	+	+	-	+	+	-	+	+	+	+	+	+	+	+	+	-	+	+	+	-	-	+	-	-	+	-	+	±	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	+	-
6PS012	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	-	-	+	-	+	+	±	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-
6PM01	+	-	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-	-	+	-	+	+	+	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-
6PM04	+	-	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	±	+	+	+	+	+	-	-	+	-	+	+	±	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-

TABLE 6 (CONTINUED)

Carbon Assimilation Tests

Isolate	Maltose	L-Erythritol	Cellobiose	Raffinose	Sucrose	D-Mannitol	Melezitose	Salicin	D-Galactose	D-Xylose	Methyl-α-D-Glucoside	Ribitol	Trehalose	L-Arabinose	D-Arabinose	D-Glucitol	Soluble Starch
6PM06	+	+	+	-	+	+	-	+	+	+	+	+	+	+	+	+	-
6PM07	+	-	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+
6PB01	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
6PB02	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+
6PB08	+	-	+	+	+	+	+	+	±	+	+	+	+	+	+	+	-
6PB015	+	-	-	+	+	+	+	+	-	+	+	+	+	+	+	-	-
7PS01	±	±	±	-	-	-	±	+	+	+	+	±	±	±	±	±	±
7PS02	-	-	-	-	+	-	+	±	-	-	-	+	+	-	-	-	+
7PS03	+	+	+	-	+	+	±	+	+	+	+	+	+	+	+	+	-
7PS04	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
7PS05	+	-	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+
7PS06	+	-	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+
7PS08	+	+	+	-	+	+	-	+	+	+	+	+	+	+	+	+	-
7PS09	+	+	+	-	+	+	-	+	+	+	+	+	+	+	+	+	-
7PS010	+	-	+	+	+	+	+	+	+	+	+	±	+	+	+	+	-
7PS013	+	±	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+
7PS10'1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+
7PS10'2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+
7PS10'3	+	-	[illegible]	-	-	-	+	-	-	-	-	[illegible]	-	-	-	-	-
7PM01	+	-	-	-	+	+	+	+	+	+	-	-	-	-	-	-	+
7PM03	+	-	±	+	+	+	+	+	+	+	+	+	+	+	-	+	+
7PM05	+	-	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-
7PB01	+	-	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-
7PB04	+	+	-	-	+	+	+	-	-	-	-	-	+	-	-	+	-
7PB07	+	-	+	+	+	+	+	+	+	+	+	-	+	-	-	+	-
7PB010	±	±	±	±	-	-	-	-	+	+	±	±	±	±	±	±	-

Isolate	L-Rhamnose	L-Sorbose	D-Ribose	Melibiose	Lactose	Glycerol	Inulin	Inositol	Ethanol	Galactitol	D-Glucose	Citric Acid	Succinic Acid	DL-Lactic Acid	2-Keto-Gluconic Acid	Glucono-δ-Lactone	Potassium Nitrate Assimilation	Ethylamine HCl Assimilation	Riboflavin Production	Starch Production
6PM06	+	±	+	-	-	+	-	-	+	-	+	-	+	-	-	-	-	-	-	-
6PM07	+	+	+	+	+	+	-	-	+	-	+	+	±	±	-	-	-	-	+	-
6PB01	+	+	+	+	+	-	-	-	[illegible]	+	+	+	-	+	-	-	-	-	-	-
6PB02	+	+	+	+	+	+	-	-	+	-	+	+	+	+	-	-	-	-	+	-
6PB08	+	+	+	-	-	+	-	-	+	-	+	+	+	[illegible]	+	+	+	-	-	-
6PB015	-	+	+	-	-	+	-	-	+	-	+	+	+	+	-	±	-	-	-	-
7PS01	±	-	-	±	±	+	-	-	±	±	+	+	[illegible]	±	-	±	-	-	-	-
7PS02	+	+	+	+	-	-	-	-	+	+	+	+	+	+	-	-	+	-	-	-
7PS03	+	+	+	-	[illegible]	+	-	-	+	-	+	-	+	-	-	-	-	-	-	-
7PS04	+	+	+	-	±	-	-	-	+	+	+	+	+	+	+	+	+	-	-	-
7PS05	+	+	+	+	+	+	-	-	+	-	+	+	+	+	-	-	-	-	+	-
7PS06	-	+	+	+	+	+	-	-	+	-	+	+	+	+	-	-	-	-	+	-
7PS08	+	+	+	-	-	+	-	-	+	-	+	-	+	-	-	-	-	-	-	-
7PS09	+	±	+	-	±	+	-	-	+	-	+	±	+	-	-	-	-	-	-	-
7PS010	+	±	±	-	+	-	-	-	+	+	+	+	+	+	+	+	+	-	-	-
7PS013	+	+	+	+	+	+	-	-	+	-	+	+	+	+	-	-	-	-	+	-
7PS10'1	±	+	+	+	+	+	-	-	+	-	+	+	+	+	-	-	-	-	+	-
7PS10'2	+	+	+	+	+	+	-	-	+	-	+	+	±	+	-	-	-	-	+	-
7PS10'3	+	+	+	-	-	[illegible]	-	-	+	-	+	+	-	-	-	-	-	-	-	-
7PM01	+	+	+	-	-	-	-	-	+	-	+	+	+	-	-	-	-	-	-	-
7PM03	-	+	+	+	+	+	-	-	+	-	+	+	+	+	-	[illegible]	-	-	+	-
7PM05	-	+	+	-	-	+	-	-	+	-	+	+	+	+	-	+	-	-	-	-
7PB01	+	+	+	-	-	+	-	-	+	-	+	+	+	-	+	+	+	-	-	-
7PB04	-	-	-	-	-	±	-	-	+	-	+	-	-	-	-	-	+	-	-	-
7PB07	+	+	-	-	+	+	-	-	+	+	+	-	-	-	+	-	+	-	-	-
7PB010	-	±	-	-	+	-	-	-	±	±	+	-	-	-	-	-	+	-	-	-

Fermentation Tests

Isolate	Maltose	D-Glucose	Lactose	Raffinose	Sucrose	Cellobiose	D-Galactose	Melibiose	Trehalose	Melezitose	Inulin	Vitamin-free Medium Growth	37C Growth	Cycloheximide Resistance	50% Glucose Growth	Arbutin Growth	Gelatin Liquefaction
6PM06	-	±	-	-	-	-	-	-	-	-	-	+	-	-	+	+	-
6PM07	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-
6PB01	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-
6PB02	-	+	-	-	-	-	±	-	-	-	-	-	-	+	+	+	-
6PB08	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-
6PB015	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-
7PS01	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
7PS02	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
7PS03	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	+	-
7PS04	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7PS05	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-
7PS06	±	+	-	-	-	-	±	-	-	-	-	-	-	+	+	+	-
7PS08	-	±	-	-	-	-	-	-	-	-	-	+	-	-	+	+	-
7PS09	-	-	-	-	-	-	-	-	-	-	-	+	-	±	+	[illegible]	-
7PS010	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7PS013	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-
7PS10'1	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-
7PS10'2	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-
7PS10'3	-	-	±	-	-	±	-	-	-	-	-	+	+	-	-	-	-
7PM01	-	+	-	±	-	±	-	-	-	-	-	-	+	-	-	-	-
7PM03	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-
7PM05	-	-	-	-	-	-	-	-	-	-	-	±	+	+	-	-	-
7PB01	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-
7PB04	-	[illegible]	-	-	-	-	-	-	-	-	-	+	-	-	-	±	-
7PB07	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	+
7PB010	-	±	-	-	±	±	-	-	-	-	-	-	-	-	-	-	-

too many questionable (±) characteristics to be keyed out, for example isolate 4PB015.

Since no special culture media were used, particularly to induce sporulation, species such as these of the genus Metchnikowia, which sporulate with difficulty, might have been present in the unidentified isolates.

To finally decide on positive identification of an organism, all data for that isolate were evaluated to rule out the possibility of any morphological, cultural or physiological discrepancies. Only then was identification considered final.

DISCUSSION

Methodology in yeast research has advanced considerably in the past twenty-five years and new techniques are being continually developed to improve the accuracy and standardization of the tests involved. Researchers are attempting to establish cultural conditions as similar as possible to those existing in the natural environment from which their samples were collected.

Standardized tests have been developed to eliminate variations in results so frequently found in the area of yeast research (Wickerham, 1951). Many factors influence the final outcome, in examining the properties of a yeast isolate. (Kreger-van Rij, 1969) Thus, one must attempt to use acceptable standard media and equipment. Preparation and amount of inoculum, incubation time and temperature can affect the final results. Evaluation and interpretation of the data obtained require further standardization for they depend to a great extent on visual observation, a rather subjective method. Improvements have been made in this area with the use of such instruments as spectrophotometers to give greater objectivity.

Identification of a yeast isolate requires information about its morphological, reproductive, cultural and biochemical characteristics. Using a biochemical key does not eliminate the necessity of acquiring information about the non-biochemical characteristics.

As regards morphology, the polyploidy of yeast cells is a source of difficulty, since there are variations in cell shape and size, not only between genera but also within a species itself. The presence of spores together with their shapes can be very characteristic, for example: the hat-shaped spores of *Hansenula*. The presence of asexual spores, of a true mycelium or pseudomycelium together with the forms taken by these structures can be some of the determining factors in identification.

The metabolism, and therefore the growth, of a yeast species is affected by a number of factors, which, in turn, influence the final identification of the organism. These factors may include: pH tolerance, reaction to antibiotics, temperature tolerance, sodium chloride tolerance, culture age and biochemical behavior. In the following discussion, since reference is made primarily to marine-occurring yeasts, the qualification will not be used. References to terrestrial forms will be so qualified.

pH Tolerance

Low pHs (3.5 to 5.5) have been suggested for many media developed for the isolation and culture of yeasts. While such pHs do help to decrease associated bacterial populations, they can also destroy acid-sensitive yeasts such as those whose natural environments is alkaline and well-buffered as is the case with sea water which has a pH range of 7.5 to 8.4. No growth determinations on low pH media were carried out in this study.

Norkrans (1966b) did an extensive study on the ability of yeasts, isolated off the west coast of Sweden, to grow at various pH ranges. Of the yeasts tested, she could discern two groups with respect to their pH optima. The first group reached maximum total growth at pH 8.1 to 8.2 or higher, despite the fact that the actual growth rate was reduced. The lag phase of growth was prolonged. This alkaline-tolerant group included Rhodotorula glutinis, Debaryomyces hansenii, Cryptococcus laurentii, Cr. luteolus, Cr. albidus and one strain of Torulopsis famata. The second group reached maximum total growth at pH 7.3 to 7.5, again after a prolonged lag phase and reduced growth rate. There was little or no growth at pH 8.1 to 8.2. This group included Cryptococcus laurentii, Cr. luteolus and Torulopsis famata, which were also represented in the first group, together with Candida parapsilosis, Rhodotorula infirmo-miniata and Saccharomyces cerevisiae, found only

in the second group. The first group would probably be better able to survive in sea water but the second group, at the higher end of its optimum pH range, could also survive. Species from both groups were isolated in the present study: *Rhodotorula glutinis*, *Debaryomyces hansenii* and *Cryptococcus albidus* of the first group and *Rhodotorula infirmo-miniata* (synonymous with *Cryptococcus infirmo-miniatus*) of the second group.

Seshadri and Sieburth (1971) tested media of various pHs to find the optima for growth of yeasts isolated from various seaweeds collected in Narragansett Bay, Rhode Island. The most appropriate pH was found to be 7.0. The yeast species examined included *Candida parapsilosis*, *C. zelanoides*, *Rhodotorula rubra* and *R. lactosa*. A modification of Seshardri and Sieburth's (1971) pH 7.0 medium was used in the present study for isolation procedures.

Antibiotics

An approach intended to reduce bacterial populations associated with yeasts has been to incorporate very high concentrations of antibiotics in the isolation media. Richards and Elliot (1966) pointed out that this procedure was not always advisable. They found that streptomycin, even at low concentrations (10 to 100 ug

per ml), inhibited the growth of many yeasts. Only *Candida utilis* was resistant to streptomycin at 100 ug per ml. Ahearn, Roth and Meyers (1968) used chloramphenicol at a concentration of 500 mg per liter in their isolation medium. No mention was made of yeast inhibition. Smith and Marchant (1968, 1969) demonstrated that chloramphenicol at a concentration of 500 mg per liter inhibited the growth of *Rhodotorula glutinis* and at higher concentrations was destructive to balanced cell wall synthesis.

Seshadri and Sieburth (1971) examined the concentration of antibiotics required to allow yeast growth and suppress bacterial growth. Penicillin, streptomycin and chlortetracycline did not effectively prevent bacterial growth at low concentrations (100 to 200 mg per liter). However, various combinations of these were found to be suitable. Chloramphenicol, by itself, at a concentration of 100 mg per liter, suppressed bacterial growth and permitted yeast growth and was, therefore, the antibiotic of choice for use in the isolation medium used in the present study.

The antibiotic activity of sea water itself must be considered when isolating yeasts from that environment. It has been found that natural sea water is more bactericidal than synthetic sea water (ZoBell, 1946). This effect is probably due to the production of anti-

biotic substances by such organisms as algae and bacteria in the sea.

Sieburth (1959, 1960, 1961) examined the antimicrobial properties of Antarctic sea water, the algae, *Phaeocystis pouchetii*, and phytoplankton and found the antibiotic substance produced by *P. pouchetii* to be acrylic acid. Seshadri and Sieburth (1971) include a diluting procedure when isolating yeasts to try to avoid the inhibitory effect to some extent.

Jakowska and Nigrelli (1960) found that sponges such as the North Atlantic red-beard sponge, *Microciona prolifera*, excreted antimicrobial substances.

Welch (1962) demonstrated the antifungal activity of substances extracted from thirty-five marine algae including species of Rhodophyta, Chlorophyta, Phaeophyta and Cyanophyta.

Buck, Meyers and Kamp (1962) reported antimicrobial activity exhibited by substances produced by a number of bacteria, isolated from various sources in Biscayne Bay, Florida and off Bimini in the Bahamas. Of the twenty species of bacteria examined, sixteen exhibited antiyeast activity.

In a later study, Buck, Ahearn, Roth and Meyers (1963)

isolated from the amphipod, *Erichthonius brasiliensis*, a *Pseudomonas* species showing definite antiyeast activity against both marine and terrestrial forms of *Rhodotorula glutinis*, *R. rubra*, *Candida tropicalis* and *Debaryomyces kloeckeri* (synonymous with *Debaryomyces hansenii*) and one terrestrial isolate of *Rhodotorula pallida*.

Olesen, Maretzki and Almodovar (1964) found at least six different antimicrobial substances from nine species of macroscopic algae collected off Puerto Rico. *Candida albicans* was inhibited by several of these substances.

Buck and Meyers (1965) examined the antiyeast activity of pelagic plankton, sponges, alcyonarians, *Pseudomonas* species and nine other genera of bacteria. Of thirty-seven plankton samples, thirteen sponges and ten alcyonaria, only three (One plankton sample, one sponge sample and one alcyonarian sample) displayed antiyeast activity. Of thirteen clinical *Pseudomonas* species examined, twelve showed some antiyeast activity against *Candida parapsilosis*, *Cryptococcus neoformans*, *Rhodotorula minuta*, *R. marina* and *R. mucilaginosa*. Two marine *Pseudomonas* isolates were inactive. Of the other genera of bacteria examined, six displayed antiyeast activity.

Temperature

In the present study, yeasts were isolated from samples

collected at temperatures ranging from -1.5C to 3.5C. The cultures were grown in the laboratory at both 4.5C and 25C. While most of the cultures grew at both temperatures, they grew much more rapidly at 25C with the exception of Leucosporidium scottii which seemed to grow at the same rate at both temperatures.

Roth, Ahearn, Fell, Meyers and Meyer (1962) isolated yeasts from various substrates from Biscayne Bay, Florida. Yeasts isolated from waters ranging in temperature from 4 to 10C grew at 6C and 24C in the laboratory. However, though the total yields were the same for both temperatures, the organisms grew much more slowly at 6C.

Phaff, Mrak and Williams (1952) examined the temperature ranges allowing growth of yeasts isolated from the washings of shrimp, Peneaus setiferus, collected in the Gulf of Mexico. Rhodotorula glutinis and Candida guilliermondii both grew at 0C. However, Rhodotorula glutinis had a temperature range of 0C to 32C, while Candida guilliermondii grew at temperatures from 0C to 38C. Rhodotorula mucilaginosa (synonymous with Rhodotorula rubra), Torulopsis albida (synonymous with Cryptococcus albidus var. albidus) and Torulopsis aeria (synonymous with Cryptococcus albidus var. aeria) all grew at temperatures ranging from 2.5C to 32C. Trichosporon diddensii (synonymous with Candida diddensii) grew in a range from 10C to 38C. Trichosporon lodderi (synonymous with Candida tropicalis) grew from 10C to 41.5C. Other

yeasts that grew at temperatures up to 41.5C included a variety of *Trichosporon cutaneum*, *Torulopsis glabrata* and one isolate of *Candida parapsilosis*. The colours of the cultures sometimes varied with the temperatures, for example, one isolate of *Rhodotorula glutinis* appeared bright yellow at 5C but pink when grown at room temperature.

Van Uden and Farinha (1958) did a study on temperature relations of strains of *Candida zeylanoides*. They found two distinct groups: one with a temperature maximum of 41C to 43C, having small cells and requiring biotin, pyridoxine and thiamine for growth; the other group having a temperature maximum of 30C to 32C with large cells and requiring only biotin for growth. These characteristics were stable throughout each group except for insignificant variations and this suggested a division into two separate species: *Candida zeylanoides* and *Candida norvegensis*.

In a later study, van Uden and Carmo-Sousa (1959) studied the temperature relations of *Candida parapsilosis* and *C. lusitaniae*. All 21 strains of *C. parapsilosis* had very similar maximum temperatures allowing growth, varying only within three degrees (40C to 43C). All six strains of *C. lusitaniae* had maximum temperatures varying only within two degrees (43C to 45C). Again, this indicates

the stability of this temperature characteristic within a species.

Cooke (1965) examined the ability of yeasts to grow at 37C. He studied 2300 strains of 70 species isolated from sewage, sewage-polluted waters and soils, sewage treatment processing plant areas, waste stabilization ponds and several other such sources. Of 16 strains of *Candida humicola* tested, 31.3% grew at 37C. Of 156 strains of *Candida tropicalis*, 97.4% grew at 37C. Of 37 strains of *Cryptococcus albidus*, 5.4% grew at 37C. One hundred and ninety-one strains of *Rhodotorula glutinis* were tested and 6.3% were found to grow at 37C. Of the three strains of *Rhodotorula graminis* tested, none grew at 37C. Three hundred and fifty strains of *Rhodotorula mucilaginosa* (synonymous with *Rhodotorula rubra*) were tested, of which 38.6% grew at 37C.

In the present study, isolates identified as *Candida humicola* grew at 37C, as did isolates of *Candida tropicalis*. Cultures of *Cryptococcus albidus* did not grow at 37C. *Rhodotorula rubra* and *R. glutinis* isolates grew at 37C but those identified as *R. graminis* did not grow at this temperature.

In the same study (Cooke, 1965), all strains of some species grew at 37C and all strains of several other species

did not grow at 37C. However, there were species with variations among the strains and Cooke proposed that this might be due to certain of the strains having become adapted to grow at 37C.

There has been some confusion about the definition of the term, "psychrophile". Generally, researchers have used this term to describe an organism having a low optimum temperature for growth. Baxter and Gibbons (1962) defined "psychrophile" as an organism reaching maximum growth at 20C or below. Morita (1975) reviewed current concepts of psychrophilism in bacteria together with the terminology involved. He has pointed out that the definition used for bacteria can also be applied to yeasts. Psychrophiles are defined by Morita (1975) as organisms having a temperature minimum of 0C or less, a temperature maximum of approximately 20C and a temperature optimum of 15C or lower.

Sinclair and Stokes (1965) isolated a number of yeasts from snow, ice, soil, sea water and marine sediments in Antarctica. They described the isolates as obligate psychrophiles growing well at 0C and having temperature optima below 20C, mainly 15C. From 67 isolates, 19 bacteria and 48 yeasts, they found four obligately psychrophilic yeasts. These included a single isolate of *Torulopsis* and three isolates of *Candida*.

Norkrans (1966b) tested 35 strains of 18 species of yeasts, isolated off the west coast of Sweden, for their ability to grow within several temperature ranges from 3C to 10C. All five strains of *Cryptococcus albidus* grew at 3C and up to 10C as did one strain of *Cr. laurentii* and an unidentified species of *Cryptococcus*. *Debaryomyces hansenii* grew at temperatures of approximately 6C to 10C. *Rhodotorula glutinis* grew in a range from 7C to 10C. *R. infirmo-miniata* (synonymous with *Cryptococcus infirmo-miniatus*) grew in a range from 6C to 10C but appeared to cease pigment production at the lower temperature. *R. rubra* grew from 7.5C to 10C but, again, appeared to lose pigmentation at the lower temperature.

Baxter and Gibbons (1962) in studying psychrophilic and mesophilic *Candida* species (*Candida* species and *C. lipolytica* respectively) found the psychrophile to be more heat-sensitive at the higher temperatures (30C to 40C). At 0C the oxidative ability of the mesophile was almost completely inhibited. There was also a striking difference between the psychrophile and the mesophile in permeability to glucosamine at the lower temperatures (0C and 10C). They suggested that this difference might be due to changes in the lipid portion of the cell membrane, as for example, closer packing of the molecules in the mesophile at lower temperatures. Kates and Baxter (1962)

studied the lipid composition of three psychrophilic yeasts (a *Candida* species isolated from grape juice and two isolates of *Candida scottii* from Antarctica) and two mesophilic yeasts (*Candida lipolytica* and *Rhodotorula glutinis*). They found that the major fatty acids in *Candida lipolytica* (grown at 25C) were (in order of descending concentration) oleic, linoleic, palmitic and palmitoleic acids. The major fatty acids of the *Candida* species (grown at 10C) were linoleic, linolenic, oleic, palmitic and palmitoleic acids. The psychrophilic species appeared to have more highly unsaturated fatty acids (1.4 to 1.7 double bonds per mole). The fatty acids of *Candida lipolytica* had 1.0 double bond per mole and those of *Rhodotorula glutinis* had 0.6 double bond per mole. Within a species there tends to be a shift toward more unsaturated fatty acids with a temperature decrease.

Sodium Chloride Tolerance

Cultures examined in the present study were isolated from plankton samples from sea water having salinities ranging from 30.3 to 38.5 °/oo. All of the isolates were able to grow on media made with distilled water.

Larsen (1962) attempted to define the various degrees of NaCl tolerance or requirement by microorganisms. He described "nonhalophiles" as organisms growing best in media

having less than 2% NaCl concentration. He divided the "halophiles" into three groups: the slightly halophilic microorganisms growing best in media of 2-5% NaCl concentration, the moderately halophilic reaching optimum growth in media containing 5-20% NaCl, and the extremely halophilic growing best in media of 20-30% NaCl concentration.

Johnson and Sparrow (1961) stated that there was no real need to use sea water media for the isolation of marine-occurring yeasts. Morris (1968) agreed with this when he found that all yeasts, isolated from the Clyde estuary, the North Sea and off the Iceland coast, grew well on distilled water media except for Debaryomyces kloeckeri (synonymous with Debaryomyces hansenii), which seemed to prefer a NaCl concentration closer to that of sea water.

Phaff, Mrak and Williams (1952), examining yeasts isolated from washings of shrimp collected in the Gulf of Mexico, found that approximately 54.8% of the isolates were quite halotolerant growing in media with NaCl concentrations of 14.7% to 16%. Only one of these isolates did not grow in the latter concentration. Rhodotorula glutinis, R. mucilaginosa (synonymous with Rhodotorula rubra), Trichosporon lodderi (synonymous with Candida tropicalis), Candida guilliermondii and C. parapsilosis grew well at the 16% NaCl concentration.

Torulopsis albida (synonymous with *Cryptococcus albidus* var. *albidus*) and *Torulopsis aeria* (synonymous with *Cryptococcus albidus* var. *aerius*) were much less halotolerant growing in NaCl concentrations of up to only 9.8%.

Ross and Morris (1962) found that *Debaryomyces* species, isolated from fish of Scottish coastal waters, exhibited greater halotolerance than did most other genera. They also examined marine-occurring yeasts versus their terrestrial counterparts. As far as halotolerance was concerned, no real distinctions could be drawn between isolates from the two environments.

Norkrans (1966b) found many halotolerant forms in her study of yeasts isolated off the west coast of Sweden. Strains of *Candida parapsilosis* and *Pichia etchellsii* grew at a NaCl concentration of 20%. Strains of *Debaryomyces hansenii* grew at NaCl concentrations of up to 24% and survived for prolonged periods at higher concentrations (28%). She found that most isolates of all species could grow in sea water of 34°/oo salinity and appeared to attain optimum growth under this condition. Most strains also withstood the higher osmotic pressures created by the introduction of NaCl into the medium.

Unlike marine bacteria which have a requirement for Na^+ ion, fungi can replace that ion with other monovalent

ions. Norkrans (1968) studied a number of terrestrial and marine-occurring isolates and found that all the yeasts, except Candida zeylanoides grew equally well in media of NaCl concentrations of 0 to 4%. This was the case even when K+ ion was substituted for all the Na+ ion in the basic growth medium. Growth in 0% NaCl concentration proved that all the yeasts examined in this study had no specific need for NaCl.

The previously mentioned studies seem to indicate that while a number of yeasts exhibit varying degrees of halotolerance, growth in NaCl solutions of high concentrations appears to be a condition of tolerance, rather than of necessity. Here, the problem of defining a "marine" yeast is encountered. Morris (1975) defined a marine yeast as one which can be isolated in pure culture from a marine environment, can also exhibit reproductive ability in such an environment and has characteristics unlike yeasts isolated from terrestrial environments. Few strictly "marine" yeasts have been isolated with the exception of several species of Metchnikowia (van Uden and Castelo-Branco, 1961). Most appear to be marine-occurring forms of already studied terrestrial species. Although many yeasts in marine waters undoubtedly originated from terrestrial sources by contamination, it could be that the techniques, particularly the use of low pH media employed up to quite recently have been

for the isolation of forms found in the sea. Some yeast species are more indigenous to marine waters than others. Debaryomyces hansenii is often the most common species identified in marine studies. Thus, in the present study, Debaryomyces hansenii constituted 29.3% of the isolates identified. This species is one of the most halotolerant of yeasts. It is thus probably well-suited to the marine environment. Other halotolerant species often isolated from marine sources, and identified in the present study, include Rhodotorula glutinis, R. rubra, Candida diddensii, C. tropicalis and Cryptococcus albidus var. albidus. However, all these species and Debaryomyces hansenii, in the present as well as in previous studies, grew well on media prepared with distilled water indicating that the NaCl of their natural habitat was not required for growth.

Age of Cultures

The cultures used in the present study were isolated from samples collected from November, 1972 to June, 1973, but it was not possible to test them biochemically until the period from March, 1974 to May, 1975. Slow growth was apparent on the YM medium alone but after addition to the medium of a 1% vitamin supplement and after several sub-culturings, vigorous growth was established and the overall appearance of the cultures improved considerably.

Lyophilization is undoubtedly the best method for the

preservation of cultures over a long period of time. Wickerham and Flickinger (1946) obtained excellent results by using this procedure to store yeasts cultures for up to four years. In most cases, they achieved 100% recovery. Most important, the reactivated organisms had the same characteristics as recorded for the original cultures.

Kreger-van Rij (1969) described some of the changes that can occur with long maintenance in culture. The colonies may change from smooth to rough with subsequent changes to more aberrant cell shapes and altered growth behavior. In the latter instance, a culture that originally formed no pellicle may develop one. The absence of a pseudomycelium may give way to its presence. Sporulating ability may be lost over a period of time. Kreger-van Rij (1969) explained this by pointing out that factors favoring sporulation undoubtedly deteriorate over time or, possibly, asporogenous forms of the yeast take over and predominate the culture. The ability to form spores can usually be reinstated, as was found in the present study by the addition of a vitamin supplement to the medium. Wickerham and Burton (1954) obtained successful results by using heat treatment in a liquid medium, the temperature of which was adjusted after determining the heat resistance of the vegetative cells of the culture.

In the present study, the original cultures which had

been maintained on YM agar for a prolonged period, gave doubtful Gram reactions, had very irregularly shaped cells, produced few or no spores, showed very pale and uneven pigmentation and were watery in consistency. The addition of a vitamin supplement restored Gram reaction, cell shape, spore formation, pigmentation and consistency to that of the original cultures.

Another change that may occur in aging cultures is a gradual transformation from the haploid to the diploid state. The predominance of diploid cells is probably due to the absence of sporulation after diploidization (Kreger-van Rij, 1969).

The longer isolates are maintained on a culture medium, the greater is the likelihood of mutation and selection of characteristics unlike those of the original. The type of culture medium is therefore of some significance. Changes can occur in the ability of a yeast to carry out such processes as fermentation and assimilation. Roberts and van der Walt (1960) demonstrated that *Saccharomyces capensis*, a non-fermentor of maltose, acquired the ability to utilize this compound after maintenance on malt agar. They found that this was caused by mutation of a single gene.

Ahearn, Roth and Meyers (1962), upon examination of

357 marine (from subtropical waters) and terrestrial strains of Rhodotorula species, noted a loss in enzymatic activity in some species after a long period of maintenance in culture. This was particularly noticeable in the ability of an isolate to assimilate maltose or lactose. Generally, they found a decline in biochemical activity in older cultures but they also found that some of the progeny differed in biochemical reactions from the parent. Thus, Rhodotorula pallida progeny were sucrose-positive and galactose-negative whereas the parent culture was sucrose-negative and galactose-positive. They suggested that such phenomena might be due to genetic instability, but also emphasized the importance of standardization of technique.

Fell and van Uden (1963) found that Candida parapsilosis (isolated from Biscayne Bay, Florida and the Bahamas area) failed to ferment glucose and galactose upon initial isolation but its normal fermentative abilities reappeared after maintenance in culture.

Scheda and Yarrow (1966) examined the instability of physiological properties used as criteria in the taxonomy of yeasts with reference to fermentation of carbon sources by lyophilized cultures and cultures maintained for long periods of time on malt agar. Revived lyophilized cultures had the same characteristics as the original cultures but all others acquired disimilar characteristics. However, no other systematic or comprehensive studies have been carried out

to describe the morphological and physiological changes occurring in a particular species of yeasts when maintained in culture over a period of time.

Biochemical Characterization

A substrate may be metabolized by a yeast cell only if the cell has the ability to convert it into an intermediary metabolite. If the substrate is not metabolized, it may be for reasons of inability to gain entrance into the cell or because the enzymes needed for conversion to an intermediary metabolite are lacking.

Assimilation

Yeasts such as Debaryomyces hansenii are capable of assimilating a variety of carbon compounds. Others are characterized by lack of this ability. Thus, some Pichia species can assimilate but a few carbon compounds. Generally speaking, yeasts possess considerable assimilative versatility. This could be quite advantageous to yeasts in the marine environment where many compounds may be available as sources of energy.

Van Uden and Fell (1968) examined the assimilation patterns of 302 species of yeasts, each represented by several strains. Of these, 17 were forms which were found to dominate in the marine environment. Thirty compounds comprising hexoses, pentoses, disaccharides, trisaccharides, polysaccharides, polyols and heteroglycosides were tested. The

average number of compounds assimilated by each strain of a species was then calculated. In aggregate, the yeasts assimilated an average of 12.8 carbon compounds. Marine-occurring yeasts assimilated an average of 19.2 carbon compounds, a considerable advantage for yeasts in such an environment.

Various techniques for detecting carbon assimilation have been devised. Beijerinck (1889) introduced an auxanographic method with plates of agar devoid of a carbon source. The organism was then streaked upon the plate, an agar overlay poured and, after solidification, small amounts of the test substrates (glucose and asparagine) were deposited on the surface of the overlay. While this test could be read in two days for some organisms, others requiring longer incubation periods could not be tested because the agar underwent hydration.

Diddens and Lodder (1942) used a modification of Beijerinck's method. Approximately 2 ml of a dense suspension of yeast cells were poured into a Petri dish. An agar medium, cooled to 40C, was then poured over this and the contents of the plate mixed immediately. After solidification, small quantities of the dry carbon compounds were deposited on the agar surface. The agar medium consisted of 2% glucose, 0.1% KH_2PO_4, 0.05% $MgSO_4$ and 2% washed agar. While Diddens and Lodder (1942) assumed that their medium contained sufficient growth factors, Wickerham and Burton (1948) found it to be somewhat

inadequate in this respect. Okunuki (1931) was the first to recommend the examination of a greater variety of carbon compounds. Sixteen compounds including pentoses, alochols and some of the more complex polysaccharides were tested using a five day incubation period. The ability of yeasts to use five organic acids, with incubation periods of 35 days, were also tested. Shifrine, Phaff and Demain (1954) developed a method of carbon assimilation testing by using the replica plating procedure of Lederberg and Lederberg (1952). They prepared plates of agar with 0.5% concentrations of the carbon source and replicated yeast growth from the original plate (containing twenty-five different yeast colonies) to plates containing the six sugars used as carbon sources. A difficulty encountered, due to excretion of intermediates into the medium, was that some strains which, for example, had been sucrose-negative at the outset became sucrose-positive. Although positive and negative reactions appeared at 2 days, the reactions were all positive at 6 days. Donkersloot (1966) developed a method of testing carbon assimilation using paper chromatography. A yeast culture was inoculated into tubes containing a basal medium each with several different carbon sources. One medium contained melibiose, maltose, sucrose, glucose, L-arabinose, ribose, rhamnose, citric acid, succinic acid and lactic acid. A second contained cellobiose, galactose, xylose, raffinose, sorbose, α-methyl-D-glucoside and salicin. A third contained dulcitol, D-arabinose, adonitol, erythritol,

glycerol, lactose and trehalose. The fourth contained melezitose, inositol, D-mannitol and sorbitol. At periods of 3, 7 and 14 days incubation, samples were withdrawn from each of the tubes and spotted on paper for descending chromatography. If the yeast in question had the ability to utilize a certain compound, this would be indicated by the gradual disappearance of that compound from the medium. One of the problems encountered in this method is the inability to know just how the utilization of one compound in the medium will affect the utilization of another in the same medium. Also, this method presents certain practical difficulties to the researcher attempting to identify hundreds of yeast cultures, in that a considerable amount of equipment is required, despite the short-cut of using several compounds in a single tube. The most suitable method developed thus far for carbon assimilation testing is that developed by Wickerham and Burton (1948). A spectrophotometric modification of the method was used in the present study. Wickerham and Burton's (1948) method uses a long incubation period (24 to 28 days) thus permitting yeasts which are slow to respond, due to the need for producing adaptive enzymes, to give a positive response. Consequently, the erroneous results obtained by workers using shorter incubation periods are eliminated. This method also uses a defined basal medium to avoid discrepancies caused by media (for example, yeast extract, peptone) containing traces of carbon sources.

Fermentation

Fermentation of sugars by yeasts results in the production of carbon dioxide and ethanol. The ability to carry out fermentation may vary from species to species within a genus or from genus to genus. Thus, some species of *Saccharomyces* ferment vigorously. On the other hand, the species of genera such as *Rhodotorula* and *Cryptococcus* are non-fermentors. Enzyme and carrier systems are required for fermentation and the presence or absence of these is genetically controlled.

Fermentative ability is a relatively stable characteristic of a species. There are, however, exceptions to this stability. Van der Walt and Tscheuschner (1956), for example, isolated a strain of *Saccharomyces capensis* (from grape must) which did not initially ferment maltose but did so after two weeks. Phaff, Miller and Shifrine (1956) also reported such delayed fermentation in species of yeasts isolated from *Drosophila*. The acquisition of the ability to ferment a sugar may be due to mutations as those described by Roberts and van der Walt (1960).

Scheda and Yarrow (1966), upon examining the stability and instability of various physiological characteristics of yeasts, found that there was a simultaneous acquisition of the ability to ferment a number of compounds. They concluded

that this was probably controlled by a single enzyme system.

Pappagiansis and Phaff (1956) suggested that if fermentation did not occur until three or four weeks after inoculation, it should be discounted and regarded as a negative reaction. They found that delayed fermentation was probably due to changes in the permeability of the cell wall with aging. In the present study, such delays did not pose a problem. If fermentation occurred at all, it occurred within one week.

Nitrogen Utilization

Yeasts are capable of using a number of nitrogen sources. Most can use ammonium sulphate, urea, asparagine or peptone, if an adequate supply of vitamins is provided. Wickerham (1946) devised a liquid assimilation test medium for examining the ability of a yeast to utilize nitrogenous compounds, which depends on the presence or absence of reductase systems.

In genera such as *Hansenula*, *Pachysolen* and *Citeromyces*, all species utilize nitrates. However, such genera as *Saccharomyces*, *Kluuveromyces*, *Pichia* and *Debaryomyces* are characterized by their inability to use nitrates.

In the present study, several species assimilated potassium nitrate including: *Candida diffluens*, *Rhodotorula*

glutinis var. *glutinis*, *Cryptococcus albidus* var. *albidus*, *Cr. infirmo-miniatus*, *Leucosporidium scottii* and several of the "black yeasts". Potassium nitrate utilization is one of the diagnostic criteria used to separate *Rhodotorula glutinis* from *R. rubra* and also *Rhodotorula glutinis* var. *glutinis* from *R. glutinis* var. *dairiensis*, the latter giving either a negative or weakly positive reaction to potassium nitrate assimilation.

Aliphatic amine nitrogen compounds can be used by some yeasts as a source of nitrogen. Some *Saccharomyces* species are unable to utilize such compounds due to the absence of monoamine oxidase activity whereby deamination takes place to form assimilable ammonia. This ability does not appear to be associated with adaptive enzyme systems (van der Walt, 1962), and thus, the characteristic is sufficiently consistent to be of considerable diagnostic value.

The ability to use ethylamine hydrochloride frequently appears to be absent in less advanced members of the phylogenetic line, but present in the more advanced ones. This ability often parallels the ability of an organism to use L(+) lysine. (van der Walt, 1962).

In the present study, ethylamine hydrochloride was seldom assimilated, except weakly, in species of *Rhodotorula*

rubra (two isolates) and in one isolate each of Candida diffluens and Cryptococcus infirmo-miniatus.

Starch Production

Extracellular starch production can be an important characteristic in yeast identification.

Lodder and Kreger-van Rij (1952) reported that starch production occurred in some Cryptococceaceae. Mager, Aschner and Leibowtiz, (1947) studied encapsulated yeasts and found that Candida heveanensis as well as many Torulopsis species produced starch. Aschner and Cury (1951) reported extracellular starch production in Candida humicola as well as in the genus Trichosporon.

In the present study, species of Candida humicola, Cryptococcus albidus var. albidus and Cr. infirmo-miniatus produced extracellular starch.

Cycloheximide Resistance

Whiffen (1948) was the first to report that yeasts varied in their resistance to cycloheximide (actidione), a chloroform - and water - soluble anticiotic produced by streptomycin-yielding cultures of Streptomyces griseus. Though the compound possesses no antibacterial activity, it is highty active against such yeasts as the pathogen,

Cryptococcus neoformans. One yeast particularly resistant to cycloheximide is *Kluyveromyces lactis*.

In the present study, it was found that strains of *Debaryomyces hansenii* and *Rhodotorula rubra* were resistant to the antibiotic.

Arbutin Splitting

Aesculin, arbutin, salicin and cellobiose are β-D-glucopyranosides and their hydrolyses are catalyzed by β-glucosidases. Cellobiose hydrolysis results in the formation of two molecules of glucose. Arbutin and salicin each break down into a phenolic compound and glucose.

Barnett, Ingram and Swain (1956) studied the hydrolysis of several β-glucosides by approximately 119 strains including *Candida tropicalis*, *Debaryomyces kloeckeri* (synonymous with *Debaryomyces hansenii*), *Rhodotorula glutinis* and *R. rubra*, all identified in the present study. They detected some hydrolysis of aesculin (which was originally used instead of arbutin) in all strains.

The results of the arbutin-splitting test in the present study are based on the color reaction of the aglucone part of the molecule. Several factors influence the outcome of such a test. These include: concentration of arbutin, pH of the medium, temperature of incubation and

presence of inhibitors, such as heavy metals, in the medium. Standardization of the test is therefore important.

In the present study, the following species utilized arbutin: *Debaryomyces hansenii*, *Leucosporidium scottii*, *Candida diddensii* and *C. diffluens* (weakly).

Vitamin-Free Medium Growth

Vitamin requirements serve as useful diagnostic criteria for yeast organisms. Wickerham (1951) introduced the use of a vitamin-free medium to test ability to grow without vitamins. This also standardized the tests used to examine this aspect of yeast metabolism.

Inability to grow in vitamin-free media is characteristic of such genera as: *Brettanomyces*, *Kloeckera*, *Hanseniaspora*, *Dekkera*, *Metschnikowia* and *Kluyveromyces*. Species of *Hanseniaspora* and *Kloeckera* require inositol and pantothenic acid as growth factors. Biotin and thiamine are required by *Dekkera*, *Metschnikowia* and *Brettanomyces*. Variations within a species may exist. Further, vitamins may not constitute absolute requirements, but may serve to stimulate growth considerably.

Van Uden and Farinha (1958) and van Uden and Carmo-Sousa (1959) examined vitamin requirement patterns in a number of yeasts. They found six strains of *Candida zeylanoides* to be

dependent on the presence of biotin. Three strains of *C. norvegensis* required biotin, thiamine and pyridoxine for growth. Twenty-one strains of *C. parapsilosis* required biotin for growth and six strains of *C. lusitaniae* were partially dependent on biotin and completely dependent on pyridoxine and thiamine. Both groups of workers felt that such vitamin requirement data could be of great value in showing taxonomic and ecological differences between species.

Ahearn and Roth (1962) examined 300 strains representing nine genera of yeasts isolated from various marine environments for their vitamin requirements, which were compared with the requirements of their terrestrial counterparts. Some species exhibited stable vitamin requirements while others had considerable strain variability. Most species required at least one growth factor, *Rhodotorula* and *Cryptococcus* species required thiamine while *Candida* species needed biotin for growth. In many cases, upon initial isolation, cultures were deficient in certain vitamins and were inhibited in growth by the addition of others to the medium. After maintenance in culture, these deficiencies lessened and the isolates became more independent of growth factors and also fermented compounds more vigorously. All marine isolates of *Candida tropicalis* required biotin and one also partially required thiamine for growth. All marine isolates of *Rhodotorula glutinis* were vitamin-independent

but *R. glutinis* var. *dairiensis* isolates required thiamine for growth. All marine isolates of *Rhodotorula graminis* were vitamin-independent. *Rhodotorula mucilaginosa* (synonymous with *R. rubra*) cultures from marine sources required thiamine as a growth factor, as did all cultures of *Cryptococcus albidus*. Marine isolates of *Debaryomyces kloeckeri* (synonymous with *D. hansenii*) required biotin and some required thiamine for growth. As far as the yeasts isolated from terrestrial sources were concerned, there appeared to be differences in vitamin requirements. Terrestrial *Candida* species required biotin and some required thiamine, niacin and pyridoxine for growth. Of the terrestrial *Cryptococcus* species, most required thiamine while some partially required biotin and para-aminobenzoic acid and two isolates were vitamin-independent. Terrestrial *Rhodotorula* species generally required thiamine but four isolates were vitamin-independent, one isolate required biotin and several required para-aminobenzoic acid. Of the terrestrial *Debaryomyces* species, all required biotin. On the whole, partial requirements for growth factors were more commonly encountered with the terrestrial forms than with the marine forms. However, no important distinctions could be noted between yeasts from the two environments.

The marine environment has been examined for the presence of vitamins. Vitamin B_{12}, thiamine, biotin and

niacin have been found to be of considerable importance in the biological productivity of sea water.

Menzel and Spaeth (1962) examined the vitamin B_{12} content of Sargasso Sea waters off Bermuda and noticed a cycle corresponding to that of primary production. They suggested that although the vitamin B_{12} may not be an essential growth factor for phytoplankton, its concentration may be a controlling factor on the species composition.

Vishniac and Riley (1961) studied the cobalamin and thiamine content of the waters of Long Island Sound. Although cobalamin concentrations somewhat reflected phytoplankton growth, thiamine was present in very small quantities and in barely detectable amounts far away from shore in the main body of the sound. They suggested that thiamine probably came from terrestrial sources by land drainage.

Natarajan and Dugdale (1966) and Natarajan (1968, 1970) carried out extensive work on the distribution of vitamin B_{12}, thiamine, biotin and niacin in northern Pacific waters off Kodiak Island and extending to the Oregon coast. They found high concentrations of thiamine above the thermocline in the water column. Further, thiamine concentrations increased during the months of high phytoplankton productivity. In the 1968 study, Natarajan found

no niacin in any of his samples. Also in the same study, thiamine appeared to be the most prevalent vitamin found being present in relatively high concentrations of up to 490 ng per liter. Biotin was found in a few locations, but in lesser quantity than thiamine. In the 1970 study, Natarajan found vitamin B_{12} in 86% of his samples and thiamine in 74%. However, yeasts are not known to require vitamin B_{12}.

In the present study, the following species were found to grow on vitamin-free medium: Candida diffluens, C. diddensii, C. humicola, Rhodotorula glutinis var. glutinis, R. graminis and Leucosporidium scottii. The property must be of considerable value to marine-occurring organisms living an an environment where certain vitamins can be found only in limited amounts during the low-productivity seasons of the year. Vitamin-free medium growth is of significant diagnostic value. Thus, it is one of the distinguishing characteristics between Rhodotorula glutinis var. glutinis and R. rubra, the latter requiring thiamine for the stimulation of growth.

Distribution

Studies have been made throughout the world indicating the universal presence of yeasts in marine waters. Species have been isolated from estuaries, coastal waters, open ocean areas, at all depths in the water column and from

marine sediments. Yeasts have also been identified from samples of various marine plants and animals and play a role in the food chain of the world's oceans. A suggestion has been made that yeasts may be involved in geochemical processes (Buck and Greenfield, 1964).

Most marine-occurring yeasts can be assigned to species commonly found in terrestrial environments. However, some new genera have been created due to their sufficiently distinctive morphology. For example, Fell (1966) isolated *Sterigmatomyces* from the Indian Ocean and Fell and Statzell (1971) isolated *Sympodiomyces* from southern Pacific, Indian and Antarctic waters.

There has been some evidence of zonation of yeast populations in marine waters. Kriss (1963) found that the numbers and types of yeast cells decreased with an increased distance from land. This may be due to the greater dilution of nutrients in open ocean waters but most researchers feel that the higher numbers in coastal waters indicate contamination from terrestrial sources. Zones of high yeast density have been reported to be associated with algal blooms. (Meyers, Ahearn, Gunkel and Roth, 1967). Kriss (1963) noted greatest concentrations of both bacteria and yeasts at the boundaries of the ocean currents. Fell and van Uden (1963) reported increased yeast numbers at the boundary of the Atlantic Ocean and the Gulf Stream, off

the Bahamas.

Thirteen species plus a number of "black yeasts" have been isolated in the present study. All have previously been isolated from marine waters. None of these species are unique to Newfoundland waters or even to northern Atlantic waters. Morris (1975), upon reviewing the literature, found that approximately 120 species of yeasts had been isolated from marine environments and among the most frequently identified were *Rhodotorula mucilaginosa* (synonymous with *Rhodotorula rubra*), *Candida tropicalis*, *Debaryomyces hansenii* and *Cryptococcus albidus*.

Many researchers have found "black yeasts" to constitute a relatively large percentage of their isolates (van Uden and Castelo Branco, 1963; Meyers, Ahearn, Gunkel and Roth, 1967). In the latter study, they were classified as *Aureobasidium pullulans*, a yeast-like fungus difficult to distinguish from certain strains of *Cryptococcus*, *Taphrnia* and *Cephalosporium*. Due to its formation of melanin pigment, it has been excluded from the yeasts. However, considering the large percentage of these "black yeasts" and their common occurrence in other studies, the data on these organisms have been tabulated in the present study. (All "black yeasts" responded well to the conditions adapted for yeast growth).

Information of the marine ecological and geographical distribution of the yeasts identified in the present study is given in Table 7. The species have been divided into 13 groups, the fourteenth group comprising the "black yeasts". All species names present in a group are synonymous with the first listed for the group. This first designated genus and species name is the accepted nomenclature according to Lodder (1970). Included under the "Sources" column are some non-marine locations but these are given because of the distinct possibility of their having an effect on the yeast populations of the surrounding marine waters.

TABLE 7

MARINE ECOLOGICAL AND GEOGRAPHICAL DISTRIBUTION

SPECIES	SOURCE	GEOGRAPHICAL LOCATION	AUTHORITY
1. *Debaryomyces hansenii:*			
D. nicotinae			
D. hansenii			
D. subglobosus			
D. kloeckeri	Sea water samples	Indian Ocean	Bhat and Kachwalla, 1955
D. kloeckeri	Estuary water samples Deep sea sediments	Biscayne Bay, Florida Bahamas area	Fell, Ahearn, Meyers and Roth, 1960
D. kloeckeri			
D. subglobosus	Sea water samples	North eastern Atlantic	Siepmann and Höhnk, 1962
D. kloeckeri	Soil samples	Key Biscayne, Florida	Capriotti, 1962a
D. kloeckeri	Water samples	Mouth of Miami River, Florida	Capriotti, 1962b
D. kloeckeri	Sea water samples	Gulf Stream, 10-40 miles off southern Florida	
	Fish intestines	Marine and coastal areas of Biscayne Bay, Florida	Roth, Ahearn, Fell, Meyers and Meyer, 1962
D. kloeckeri	Sea water samples	Gulf Stream near the Bahamas	
	Estuarine Sediments	The Bahamas	
	Fish samples	Biscayne Bay, Florida	Fell and van Uden, 1963
D. kloeckeri	Fish samples	Clyde estuary, North Sea, waters off the east coast of Iceland	Ross and Morris, 1965
D. kloeckeri			
D. subglobosus	Glacier samples	Ross Island, Antarctica	DiMenna, 1966
D. hansenii	Sea water samples	West coast of Sweden	Norkrans, 1966a
D. hansenii	Sea water samples	Indian Ocean and Island of Mauritius area	Fell, 1967

TABLE 7 (CONTINUED)

MARINE ECOLOGICAL AND GEOGRAPHICAL DISTRIBUTION

SPECIES	SOURCE	GEOGRAPHICAL LOCATION	AUTHORITY
D. hansenii	Sea water samples	Black Sea	Meyers, Ahearn and Roth, 1967a
D. hansenii	Sea water samples	North Sea	Meyers, Ahearn, Gunkel and Roth, 1967b
D. hansenii	Water samples	Estuarine and everglade areas of southern Florida	Ahearn, Roth and Meyers, 1968
D. hansenii	Fish samples	Clyde estuary and North Sea	Bruce and Morris, 1973
D. hansenii	Spiny lobster (*Panulirus argus*), conch (*Strombus gigas*), sand and sediments	Abaco Island, the Bahamas	Volz, Jerger, Wurzburger and Hiser, 1974
3. Rhodotorula rubra:			
R. mucilaginosa	Surface washings of shrimp	Arkansas Bay, Texas	Phaff, Mrak and Williams, 1952
R. mucilaginosa			
R. rubra	Little-neck clam	Japan	Kobayashi, Tsubaki and Soneda, 1953
R. mucilaginosa (several varieties)	Plankton and sea water samples	Black Sea, Sea of Okhotsk, North west Pacific	Kriss and Novozhilova, 1954
R. mucilaginosa	Soil samples	Antarctica	Soneda, 1961
R. mucilaginosa			
R. rubra	Sea water samples	Northeastern Atlantic	Siepmann and Höhnk, 1962
R. mucilaginosa	Sea water samples	North Pacific off Japan and East China Sea	Shinano, 1962
R. mucilaginosa	Rotted seaweeds	Japanese coastal areas	Suehiro and Tomiyasu, 1962
R. mucilaginosa	Plankton samples	Japanese coastal areas	Suehiro, Tomiyasu and Tanaka, 1962
R. mucilaginosa	Estuarine water samples	Biscayne Bay, Florida	
R. rubra	Open ocean waters Fish intestines	Gulf Stream off Florida Biscayne Bay, Florida	Roth, et al., 1962
R. mucilaginosa	Estuarine water samples Open ocean waters Deep-sea sediments Fish samples	Biscayne Bay, Florida Gulf Stream off Florida Andros Island, the Bahamas Biscayne Bay, Florida	Fell and van Uden, 1963

TABLE 7 (CONTINUED)

MARINE ECOLOGICAL AND GEOGRAPHICAL DISTRIBUTION

SPECIES	SOURCE	GEOGRAPHICAL LOCATION	AUTHORITY
R. rubra	Water samples	Rivers Tagus and Sado, Portugal and adjacent Atlantic Ocean	Taysi and van Uden, 1964
R. mucilaginosa			
R. rubra	Fish samples	North Sea, Icelandic coastal waters and Clyde estuary	Ross and Morris, 1965
R. mucilaginosa	Glacier samples	Ross Island, Antarctica	DiMenna, 1966
R. rubra	Sea water samples	West coast of Swedon	Norkrans, 1966a
R. rubra	Sea water samples	Indian Ocean and Island of Mauritius area	Fell, 1967
R. rubra	Sea water samples	Black Sea	Meyers et al., 1967a
R. rubra	Sea water samples	North Sea	Meyers et al., 1967b
R. rubra	Water samples	Miami River estuary, Everglades region and Lake Okeechobee in Southern Florida	Ahearn et al., 1968
R. rubra	Sediments, soil samples and stream samples	Lake Vanda and Lake Miers, Antarctica	Goto, Sugiyama and Iizuka, 1969
R. rubra	From areas of oyster grass, *Spartina alterniflora*	Barataria Bay, Louisiana	Meyers, Nicholson, Rhee, Miles and Ahearn, 1970
R. rubra	Sea water samples	Long Island Sound	Combs, Murchelano and Jurgen, 1971
R. mucilaginosa	Fish samples	Clyde estuary and North Sea	Bruce and Morris, 1973
R. rubra	Spiny lobster (*Panulirus argus*), conch (*Strombus gigas*), sand and sediments	Abaco Island, the Bahamas	Volz et al., 1974
R. Rhodotorula glutinis:			
R. glutinis	Washings of shrimp	Arkansas Bay, Texas	Phaff et al., 1952
R. glutinis	Plankton and sea water samples	Sea of Okhotsk and Northwest Pacific	Kriss and Novozhilova, 1954
R. glutinis	Sea water samples Deep sea sediments	Biscayne Bay, Florida Bahamas	Fell et al., 1960
R. glutinis	Sea water samples	Northeastern Atlantic	Siepmann and Höhnk, 1962
R. glutinis	Sea water samples	Northwestern Pacific	Shinano, 1962

TABLE 7 (CONTINUED)

MARINE ECOLOGICAL AND GEOGRAPHICAL DISTRIBUTION

SPECIES	SOURCE	GEOGRAPHICAL LOCATION	AUTHORITY
N. glutinis	Plankton samples	Japanese waters	Suehiro et al., 1962
N. glutinis	Soil samples	Key Biscayne, Florida	Capriotti, 1962a
N. glutinis	Water samples	Mouth of Miami River	Capriotti, 1962b
N. glutinis	Sea water samples	Northern Biscayne Bay, Florida	Roth et al., 1962
N. glutinis	Subsurface sea water samples	Southern California	van Uden and Castelo Branco, 1963
N. glutinis var *rubescens*			
N. rufula	Sea water samples	Black Sea and Sea of Okhostk	Kriss, 1963
N. glutinis	Estuarine water samples	Biscayne Bay, Florida	
	Open ocean water samples	Gulf Stream, Bimini, the Bahamas	
	Coastal waters	LaJolla, California	
	Marine sediments	Biscayne Bay, Andros Island, Bimini	Fell and van Uden, 1963
N. glutinis	Water samples	Estuaries of Rivers Tagus and Sado; Portugal and adjacent Atlantic Ocean	Taysi and van Uden, 1964
N. glutinis var *rubescens*	Fish samples	Clyde estuary, North Sea and Icelandic coast	Ross and Morris, 1965
N. glutinis	Soil samples	East Greenland	DiMenna, 1966
N. glutinis	Sea water samples	West coast of Sweden	Norkrans, 1966a
N. glutinis	Sea water samples	Indian Ocean and area of Island of Mauritius	Fell, 1967
N. glutinis	Seawater samples	Black Sea	Meyers et al., 1967a
N. glutinis	Water samples	Miami River Estuary and Everglades area, South Florida	Ahearn et al., 1968
N. glutinis	Water samples Brine shrimp Brine shrimp eggs	Great Salt Lake, Utah Salt Lake in Saskatchewan California	Lodder, 1970
N. glutinis	Water samples	Long Island Sound	Combs et al., 1971
N. glutinis	Fish samples	North Sea	Bruce and Morris, 1973

TABLE 7 (CONTINUED)

MARINE ECOLOGICAL AND GEOGRAPHICAL DISTRIBUTION

SPECIES	SOURCE	GEOGRAPHICAL LOCATION	AUTHORITY
R. glutinis	Spiny lobster (*Panulirus argus*), conch (*Strombus gigas*), sand and sediments	Abaco Island, the Bahamas	Volz et al., 1974
4. *Rhodotorula graminis:*			
R. graminis	Sea water samples	Biscayne Bay, Florida	Fell et al., 1960
R. graminis	Soil samples	McMurdo Sound, Antarctica	DiMenna, 1960
R. graminis	Subsurface sea water samples	Southern California	van-Uden and Castelo Branco, 1963
R. graminis	Estuarine water samples marine sediments	Biscayne Bay, Florida	Fell and van Uden, 1963
R. graminis	Glacier samples	Ross Island, Antarctica	DiMenna, 1966
R. graminis	Sea water samples	Indian Ocean and area of Island of Mauritius	Fell, 1967
R. graminis	Sea water samples	Black Sea	Meyers et al., 1967a
5. *Rhodotorula pallida*			
R. pallida	Plankton and sea water samples	Black Sea, Sea of Okhostk Northwestern Pacific	Krisa and Novozhilova, 1954
R. pallida	Sea water samples	Indian Ocean	Bhat, Kachwalla and Hody, 1955
R. pallida	Glacier samples	Ross Island, Antarctica	DiMenna, 1966
R. pallida	Sea water samples	Indian Ocean and area of Island of Mauritius	Fell, 1967
6. *Candida tropicalis*			
Trichosporon loddari	Lateral plate of darkened and possibly diseased shrimp	Gulf of Mexico	Phaff et al., 1952
C. tropicalis	Sea water samples	Indian Ocean	Bhat and Kachwalla, 1954
C. tropicalis	Sea water samples	Biscayne Bay, Florida	Fell et al., 1960
C. tropicalis	Soil samples	Key Biscayne, Florida	Capriotti, 1962a
C. tropicalis	Water samples	Mouth of Miami River, Florida	Capriotti, 1962b
C. tropicalis	Rotted seaweeds	Japanese coastline	Suehiro and Tomiyasu, 1962

TABLE 7 (CONTINUED)

MARINE ECOLOGICAL AND GEOGRAPHICAL DISTRIBUTION

SPECIES	SOURCE	GEOGRAPHICAL LOCATION	AUTHORITY
C. tropicalis	Plankton samples	Japanese waters	Suehiro *et al.*, 1962
C. tropicalis	Fish samples	Biscayne Bay and Bimini, the Bahamas	Roth *et al.*, 1962
C. tropicalis	Digestive tract of gull	Southern California	van Uden and Castelo Branco, 1963
C. tropicalis	Water samples Estuarian sediments Fish samples	Biscayne Bay and Bimini, the Bahamas	Fell and van Uden, 1963
C. tropicalis	Intestinal tracts of gulls and terns	Portugal	Kawakita and van Uden, 1965
C. tropicalis	Sea water samples	Indian Ocean	Fell, 1967
C. tropicalis	Sea water samples	Black Sea	Meyers *et al.*, 1967a
C. tropicalis	Sea water samples	North Sea	Meyers *et al.*, 1967b
C. tropicalis	Water samples	Southern Florida area	Ahearn *et al.*, 1968
C. tropicalis	Water samples	Long Island Sound	Combs *et al.*, 1971
C. tropicalis var *lambica*	Fish samples	Clyde estuary	Bruce and Morris, 1973
7. *Candida diddensii*			
Trichosporon diddensii	Washings of shrimp	Gulf of Mexico	Phaff *et al.*, 1952
Trichosporon atlanticum	Sea water samples	North eastern Atlantic	Siepmann and Höhnk, 1962
Candida atmosphaerica			
C. polymorpha	Sea water samples	Indian Ocean	Fell, 1967
C. diddensii			
C. polymorpha	Sea water samples	Black Sea	Meyers *et al.*, 1967a
C. diddensii	Sea water samples	North Sea	Meyers *et al.*, 1967b
C. diddensii			
C. polymorpha	Water samples	Southern Florida Everglades and coastal waters	Ahearn *et al.*, 1968
C. diddensii	Spiny lobster (*Panulirus argus*), conch (*Strombus gigas*), sand and sediments	Abaco Island, the Bahamas	Volz *et al.*, 1976

TABLE 7 (CONTINUED)

MARINE ECOLOGICAL AND GEOGRAPHICAL DISTRIBUTION

SPECIES	SOURCE	GEOGRAPHICAL LOCATION	AUTHORITY
8. *Candida humicola*			
C. humicola	Little-neck clam	Japan	Kobayashi et al., 1953
C. humicola	Water samples	Lake Vanda, Antarctica	Goto et al., 1969
9. *Candida maritima*			
Trichosporon maritimum	Sea water samples	Northeastern Atlantic	Siepmann and Höhnk, 1962
Candida maritima	Spiny lobster (*Panulirus argus*), conch (*Strombus gigas*), sand and sediments	Abaco Island, the Bahamas	Volz et al., 1974
10. *Candida diffluens*	Water samples and sediments	Lake Miers and Lake Vanda, Antarctica	Goto et al., 1969
11. *Cryptococcus albidus*			
Torulopsis albida	Surface washings of shrimp	Arkansas Bay, Texas	Phaff et al., 1952
Cr. albidus	Sea water samples Deep sea sediments	Biscayne Bay, Florida Bahamas	Fell et al., 1960
Cr. mucorugosus	*Laminaria* species	Labrador fiord, Newfoundland	Hagen and Rose, 1961
Cr. albidus	Soil samples	Antarctica	Soneda, 1961
Cr. albidus	Water samples	Mouth of Miami River, Florida	Capriotti, 1962b
Cr. albidus	Sea water samples	North Pacific off Japan	Shinano, 1962
Cr. albidus	Rotted seaweeds	Japan	Suehiro and Tomiyasu, 1962
Cr. albidus	Sea water samples	North eastern Atlantic	Siepmann and Höhnk, 1962
Cr. albidus	Plankton samples	Japanese waters	Suehiro et al., 1962
Cr. albidus	Sea water samples	Soldier Key, Florida	Roth et al., 1962
Cr. albidus	Sea water samples	Coastal waters off LaJolla, California	
	Marine sediments	Bahamas	Fell and van Uden, 1963
Cr. albidus	Sea water samples	Southern California waters	van Uden and Castelo Branco, 1963

TABLE 7 (CONTINUED)

MARINE ECOLOGICAL AND GEOGRAPHICAL DISTRIBUTION

SPECIES	SOURCE	GEOGRAPHICAL LOCATION	AUTHORITY
Cr. albidus	Water samples	Rivers Tagus and Sado, Portugal and adjacent Atlantic Ocean	Taysi and van Uden, 1964
Torulopsis pseudaria	Fish samples	North Sea	Ross and Morris, 1965
Cr. albidus	Sea water samples	West coast of Sweden	Norkrans, 1966a
Cr. albidus	Glacier samples	Ross Island, Antarctica	DiMenna, 1966
Cr. albidus	Sea water samples	Indian Ocean	Fell, 1967
Cr. albidus	Sea water samples	Black Sea	Meyers et al., 1967a
Cr. albidus	Water samples	Miami River estuary and Everglades region, Florida	Ahearn et al., 1968
Cr. albidus	Water samples	Lake Vanda, Antarctica	Goto et al., 1969
Cr. albidus	Oyster grass, *Spartina alterniflora*	Barataria Bay, Louisiana	Meyers et al., 1970
Cr. albidus	Brine shrimp	Salt Lake in Saskatchewan Canada	
	Water samples	Great Salt Lake, Utah	Lodder, 1970
Cr. albidus	Spiny lobster (*Panulirus argus*), conch (*Strombus gigas*), sand and sediments	Abaco Island, the Bahamas	Volz et al., 1974
17. *Cryptococcus infirmo-miniatus*			
Rhodotorula glutinis var *infirmo-miniata*	Plankton and sea water samples	Black Sea, Sea of Okhotsk northern Pacific Ocean	Kriss and Novozhilova, 1954
Rhodotorula glutinis var *infirmo-miniata*	Water samples	Rivers Tagus and Sado, Portugal and adjacent Atlantic Ocean	Taysi and van Uden, 1964
Rhodotorula infirmo-miniata	Sea water samples	West coast of Sweden	Norkrans, 1966a
Rhodotorula glutinis var *infirmo-miniata*	Sea water samples	Indian Ocean and Island of Mauritius area	Fell, 1967
Rhodotorula infirmo-miniata	Sea water samples	Black Sea	Meyers et al., 1967a

TABLE 7 (CONTINUED)

MARINE ECOLOGICAL AND GEOGRAPHICAL DISTRIBUTION

	SPECIES	SOURCE	GEOGRAPHICAL LOCATION	AUTHORITY
	Rhodotorula infirmo-miniata	Water samples	Everglades region of southern Florida	Ahearn et al., 1968
	Rhodotorula infirmo-miniata	Fish samples	Clyde estuary	Bruce and Morris, 1973
	Cryptococcus infirmo-miniatus	Spiny lobster (*Panulirus argus*), conch (*Strombus gigas*) sand and sediments	Abaco Island, the Bahamas	Volz et al., 1974
23.	*Leucosporidium scottii*			
	Candida scottii	Soil samples	McMurdo Sound, Antarctica	DiMenna, 1960
	Candida scottii	Glacier samples	Ross Island, Antarctica	DiMenna, 1966
	Leucosporidium scottii	Sea water samples	Antarctic Sea	Fell, Statzell, Hunter and Phaff, 1969
24.	"Black yeasts"			
	"Black yeasts"	Sea water samples	Biscayne Bay, Florida	Fell et al., 1960
	Aureobasidium pullulans	Soil samples	Key Biscayne, Florida	Capriotti, 1962a
	Aureobasidium pullulans	Water samples	Mouth of Miami River, Florida	Capriotti, 1962b
	"Black yeasts"	Sea water samples	Torres Strait	van Uden and ZoBell, 1962
	"Black yeasts"	Plankton samples	Japanese waters	Suehiro et al., 1962
	"Black yeasts"	Sea water samples	Coastal waters off LaJolla, California and open ocean waters off Bimini, the Bahamas	Fell and van Uden, 1963
	"Black yeasts"	Sea water samples	Southern California waters	van Uden and Castelo Branco, 1963
	"Black yeasts"	Water samples	Rivers Tagus and Sado, Portugal and adjacent Atlantic Ocean	Taysi and van Uden, 1964

TABLE 7 (CONTINUED)

MARINE ECOLOGICAL AND GEOGRAPHICAL DISTRIBUTION

SPECIES	SOURCE	GEOGRAPHICAL LOCATION	AUTHORITY
Aerobasidium pullulans	Sea water samples	Black Sea	Meyers et al., 1967a
Aerobasidium pullulans	Sea water samples	North Sea	Meyers et al., 1967b
"Black yeasts"	Sea water samples	Long Island Sound	Combs et al., 1971

SUMMARY

1. Yeasts were isolated from plankton samples collected at various depths off Small Point, Avalon Peninsula, Newfoundland.

2. Morphological, reproductive, cultural and biochemical characteristics were recorded for all cultures.

3. A key developed by Barnett and Pankhurst (1974), using biochemical characteristics as diagnostic criteria, was used to identify the isolates.

4. Thirteen species of yeasts were identified (in descending order of ~~frequency~~ of occurrence): *Debaryomyces hansenii*, *Rhodotorula rubra*, *Candida diddensii*, *Rhodotorula glutinis* var. *glutinis*, *Candida diffluens*, *Candida tropicalis*, *Rhodotorula graminis*, *Cryptococcus albidus* var. *albidus*; *Cryptococcus infirmo-miniatus*, *Candida humicola*, *Candida maritima* and *Leucosporidium scottii*. Several black yeasts were also isolated. None of these yeasts were unique to Newfoundland or northern marine waters.

5. The results of the various tests were discussed with reference to the seasonal and vertical distribution of the species.

6. The methodology used in the present study was discussed as well as the effects of various environmental factors on yeast growth with emphasis on the species identified.

7. The marine distribution of the identified species was reviewed.

BIBLIOGRAPHY

Ahearn, D.G. 1964. A comparative physiological and morphological study of terrestrial and marine-occurring carotenogenic yeasts. Ph.D. Thesis: Univ. Miami. 164p.

Ahearn, D.G., and F.J. Roth, Jr. 1962. Vitamin requirements of marine-occurring yeasts. Dev. Ind. Microbiol. 3: 163-173.

Ahearn, D.G., and F.J. Roth, Jr. 1966. Physiology and ecology of psychotrophic carotenogenic yeasts. Dev. Ind. Microbiol. 7: 301-309.

Ahearn, D.G., F.J. Roth, Jr., and S.P. Meyers. 1962. A comparative study of marine and terrestrial strains of *Rhodotorula*. Can. J. Microbiol. 8: 121-132.

Ahearn, D.G., F.J. Roth, Jr., and S.P. Meyers. 1968. Ecology and characterization of yeasts from acquatic regions of South Florida. Mar. Biol. 1: 291-308.

Ahearn, D.G., F.J. Roth, Jr., J.W. Fell, and S.P. Meyers. 1960. Use of shaken cultures in the assimilation test for yeast identification. J. Bacteriol. 79: 369-371.

Almeida, F.P.de. 1939. Mycologia Medica. Comp. Melhoramentos, Sao Paulo. 120p.

Anderson, H.W. 1917. Yeast-like fungi of the human intestinal tract. J. Infect. Dis. 21: 341-386.

Aschner, M., and A. Cury. 1951. Starch production in the genus *Trichosporon*. J. Bacteriol. 62: 350-352.

Aschner, M., J. Mager, and J. Leibowitz. 1945. Production of extracellular starch in cultures of capsulated yeasts. Nature. 156: 295.

Barnett, J.A., and R.J. Pankhurst. 1974. A new key to the yeasts. North-Holland Publ. Co., Amsterdam. 273p.

Barnett, J.A., M. Ingram, and T. Swain. 1956. The use of B-glucosides in classifying yeasts. J. Gen. Microbiol. 15: 529-555.

Baxter, R.M., and M.E. Gibbons. 1962. Observations on the physiology of psychrophilism in a yeast. Can. J. Microbiol. 8: 511-517.

Beijerinck, M.W. 1889. L'auxanographie ou la methode de l'hydrodiffusion dans la gelatine apliquce aux recherches microbiologiques. Arch. Neerl. Sci. 23: 367-372.

Berkhout, C.M. 1923. De schimmelgeslachten *Monilia*, *Oidium*, *Oospora* en *Torula*. Ph.D. Thesie. Utrecht. 153p.

Bhat, J.V., and M. Kachwalla. 1954. Marine yeasts off the Indian coast. Indian Acad. Sci. Proc. 41: 9-15.

Bhat, J.V., M. Kachwalla, and B.N. Mody. 1955. Some aspects of the nutrition of marine yeasts and their growth. J. Sci. Ind. Res. (India), C. 14: 24-27.

Bruce, J., and E.O. Morris. 1973. Psychrophilic yeasts isolated from marine fish. Antonie van Leeuwenhoek. 39: 331-339.

Buck, J.D., and L.J. Greenfield. 1964. Calcification in marine-occurring yeasts. Bull. Mar. Sci. Gulf Caribb. 14: 239-245.

Buck, J.D., and S.P. Meyers. 1965. Antiyeast activity in the marine environment. I Ecological considerations. Limnol. Oceanogr. 10: 385-391.

Buck, J.D., S.P. Meyers, and K.M. Kamp. 1962. Marine bacteria with antiyeast activity. Science. 138: 1339-1340.

Buck, J.D., D.G. Ahearn, F.J. Roth, Jr., and S.P. Meyers. 1963. Inhibition of yeasts by a marine bacterium. J. Bacteriol. 85: 1132-1135.

Capriotti, A. 1962a. Yeasts of the Miami, Florida area. I From Key Biscayne soils. Arch. Mikrobiol. 41: 142-146.

Capriotti, A. 1962b. Yeasts of the Miami, Florida area. II From the Miami River. Arch. Mikrobiol. 41: 147-153.

Castellani, A. 1910. Tropical bronchomycosis. Observations on a new species of epidermophyton found in tinea cruris. A new intestinal spirillum. Phillipine J. Sci., B. 5: 197-209.

Combs, T.J., R.A. Murchelano, and F. Jurgen. 1971. Yeasts isolated from Long Island Sound. Mycologia. 63: 178-181.

Conn, H.J. (ed.). 1957. Manual of microbiological methods. McGraw Hill Book Co., New York. 315p.

Cooke, W.B. 1965. The growth of yeasts at 37C. Mycopathol. Mycol. Appl. 25: 195-200.

Diddens, H.A., and J. Lodder. 1942. Die anaskosporogenen Hefen, II Halfte. North-Holland Publ. Co., Amsterdam. 312p.

Donkersloot, J.A. 1966. Utilization of mixtures of carbon compounds by yeasts. Antonie van Leeuwenhoek. 32: 419-428.

Dyer, F.E. 1947. Microorganisms from Atlantic cod. J. Fish. Res. Board Can. 7: 128-136.

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

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

Fell, J.W. and S.A. Meyer. 1967. Systematics of yeasts species in the *Candida parapsilosis* group. Mycopathol. Mycol. Appl. 32: 177-193.

Fell, J.W., and A.C. Statzell. 1971. *Sympodiomyces* gen. n., a yeast-like organism from southern marine waters. Antonie van Leeuwenhoek. 37: 359-367.

Fell, J.W., and M. van Uden. 1963. Yeasts in marine environments, p. 329-240. In C.H. Oppenheimer (ed.), Symposium on marine microbiology. Charles C. Thomas Publ., Springfield, Illinois.

Fell, J.W., D.G. Ahearn, S.P. Meyers, and F.J. Roth, Jr. 1960. Isolation of yeasts from Biscayne Bay, Florida and adjacent benthic area. Limnol. Oceanogr. 5: 366-371.

Fell, J.W., A.C. Statzell, I.L. Hunter, and H.J. Phaff. 1969. *Leucosporidium* gen. n., the heterobasidiomycetous stage of several yeasts of the genus *Candida*. Antonie van Leeuwenhoek. 35: 433-462.

Fischer, B., and C. Brebeck. 1894. Zur Morphologie, Biologie und Systematik der Kahmpilze, der *Monilia candida* und des Soorerregers. J. Cramer, Weinheim. 115p.

Fresenius, G. 1863. *Cryptococcus glutinis* Fresen. Beitrage zur Mycologie. p. 77-80.

Goto, S., J. Sugiyama, and H. Iizuka. 1969. A taxonomic study of Antarctic yeasts. Mycologia. 61: 748-774.

Hagan, P.O., and A.H. Rose. 1961. A psychrophilic *Cryptococcus*. Can. J. Microbiol. 7: 287-294.

Harrison, F.C. 1928. A systematic study of some *Torulae*. Trans. Roy. Soc. Can. 22: 187-225.

Hasegawa, T., and I. Banno. 1964. *Rhodotorula infirmo-miniata* (Okunuki) comb. n. Antonie van Leeuwenhoek 30: 65-67.

Hunter, A.C. 1920. A pink yeast causing spoilage in oysters. U.S. Dept. Agric. Bull. 819. 24p.

Issatchenko, B. 1914. Studies of bacteria of the Arctic Ocean. Trudy murmansk. nauch. Prom. Ekspedit, 1906. St. Petersburg. 75p.

Jakowska, S., and R.F. Nigrelli. 1960. Antimicrobial substances from sponges. Amn. N.Y. Acad. Sci. 90: 913-916.

Johnson, T.W., and F.K. Sparrow. 1961. Fungi in oceans and estuaries. J. Cramer, Weinheim. 688p.

Kates, M., and R.M. Baxter. 1962. Lipid composition of mesophilic and psychrophilic yeasts (*Candida* species) as influenced by environmental temperature. Can. J. Biochem. Physiol. 40: 1213-1227.

Kawakita, S., and N. van Uden. 1965. Occurrence and population densities of yeast species in the digestive tracts of gulls and terns. J. Gen. Microbiol. 39: 125-129.

Klocker, A. 1909. Deux nouveaux genres de la famille des *Saccharomyces*. C. R. Trav. Lab. Carlsberg. 7: 273-278.

Kobayaski, Y., K. Tsubaki, and M. Soneda. 1953. Marine yeasts isolated from little-neck clam. Tokyo Nat. Sci. Mus. Bull. 33: 47-53.

Kreger-van Rij, N.J.W. 1962. The use of biochemical criteria in the taxonomy of yeasts. Symp. Soc. Gen. Microbiol. 12: 196-211.

Kreger-van Rij, N.J.W. 1969. Taxonomy and systematics of yeasts, p. 5-78. In A.H. Rose and J.S. Harrison (eds.), The Yeasts, Vol I. Academic Press, London.

Kriss, A.E. 1963. Marine microbiology (Deep Sea). (Translated from Russian by J.M. Shewan and Z. Kabata). Interscience Publ., New York. 536p.

Kriss, A.E., and M.I. Novozhilova. 1954. Are yeast organisms inhabitants of seas and oceans? (Transl. from Russian by J.S. ZoBell). Mikrobiologiya. 23: 669-683.

Kudriavzev, V.I. 1954. Die Systematik der Hefen. (German transl. from Russian). Akademu Verlag, Berlin. 173p.

Larsen, H. 1962. Halophilism, p. 297-342. In I.C. Gunsalus and R.Y. Stanier (eds.), The bacteria, Vol. 4. Academic Press, New York.

Lederberg, J., and E.M. Lederberg. 1952. Replica plating and indirect selection of bacterial mutants. J. Bacteriol. 63: 399-406.

Lodder, J. 1934. Die anaskosporogenen Hefen, I Halfte. Verh. Kon. Akad. Wetenschap Sect. II, 32: 1 - 256.

Lodder, J. 1970. The yeasts, a taxonomic study. North-Holland Publ. Co., Amsterdam. 1385p.

Lodder, J., and N.J.W. Kreger-van Rij. 1952. The yeasts, a taxonomic study. North-Holland Publ. Co., Amsterdam. 713p.

Lyman, J., and R.W. Fleming. 1940. Composition of sea water. J. Mar. Res. 3: 134-146.

Mager, J., and M. Aschner. 1947. Biological studies on capsulated yeasts. J. Bacteriol. 53: 283-295.

Menna, M. di. 1958. Two new species of yeasts from New Zealand. J. Gen. Microbiol. 18: 269-272.

Menna, M. di. 1960. Yeasts from Antarctica. J. Gen. Microbiol. 23: 295-300.

Menna, M. di. 1960. Yeasts in Antarctic soils. Antonie van Leeuwenhoek. 32: 29-38.

Menzel, D.W., and J.P. Spaeth. 1962. Occurrence of vitamin B12 in the Sargasso Sea. Limnol. Oceanogr. 7: 151-154.

Meyers, S.P., D.G. Ahearn, and F.J. Roth, Jr. 1967. Mycological investigations of the Black Sea. Bull. Mar. Sci. 17: 576-596.

Meyers, S.P., D.G. Ahearn, W. Gunkel, and F.J. Roth, Jr. 1967. Yeasts from the North Sea. Mar. Biol. 1: 118-123.

Meyers, S.P., M.L. Nicholson, J. Rhee, P. Miles, and D.G. Ahearn. 1970. Mycological studies in Barataria Bay, Louisiana, and biodegradation of oyster grass, *Spartina alterniflora*. Special sea grant issue, Coastal Studies Bull. p. 111-124.

Miranda, L. Rodrigues de, and B. Norkrans. 1968. *Candida suecica* sp. n. isolated from the marine environment. Antonie van Leeuwenhoek. 34: 115-118.

Morita, R.Y. 1975. Psychrophilic bacteria. Bacteriol. Rev. 39: 144-167.

Morris, E.D. 1968. Yeasts of marine origin. Oceanogr. Mar. Biol. Annu. Rev. 6: 201-230.

Morris, E.D. 1975. Yeasts from the marine environment. J. Appl. Bacteriol. 38: 211-223.

Moskovits, G. 1951. Preliminary studies on some aspects of bacteria-plankton relations in Vineyard Sound. M.Sc. Thesis. Univ. Rhode Island. 115p.

Moskovits, G., and A. Flanagan. 1967. Growth of marine bacteria on some organic acids and its application to the selective isolation of *Pseudomonads*. Can. J. Microbiol. 13: 1561-1563.

Nadson, G., and G. Burgwitz. 1931. Hefen des Nordlichen Eismeeres. Dokl. Akad. Nauk SSSR. 4: 103-110.

Nakase, T., and K. Komagata. 1968. Taxonomic significance of base composition of yeast DNA. A News Letter for Persons Interested in Yeasts. 17: 4-7.

Natarajan, K.V. 1968. Distribution of thiamine, biotin and niacin in the sea. Appl. Microbiol. 16: 366-369.

Natarajan, K.V. 1970. Distribution and significance of vitamin B_{12} and thiamine in the subarctic Pacific Ocean. Limnol. Oceanogr. 15: 655-659.

Natarajan, K.V., and R.C. Dugdale. 1966. Bioassay and distribution of thiamine in the sea. Limnol. Oceanogr. 11: 621-629.

Norkrans, B. 1966a. On the occurrence of yeasts in an estuary off the Swedish west coast. Sv. Bot. Tidskr. 60: 463-482.

Norkrans, B. 1966b. Studies on marine-occurring yeasts: growth related to pH, NaCl concentration and temperature. Arch. Mikrobiol. 54: 374-392.

Norkrans, B. 1968. Studies on marine-occurring yeasts: respiration, fermentation and salt tolerance. Arch. Mikrobiol. 62: 358-372.

Novak, E.K., and J. Zsolt. 1961. A new system proposed for yeasts. Acta. Bot. Acad. Sci. Hungary. 7: 93-145.

Okunuki, K. 1931. Beitrage zue Kenntnis der rosafarbigen Sprosspilze. (Transl. from Japanese) Jpn. J. Bot. 5: 285-322.

Olesen, P.E., A. Maretzki, and L.A. Almodovar. 1964. An investigation of antimicrobial substances from marine algae. Bot. Mar. 6: 224-232.

Pappagiansis, D., and H.J. Phaff. 1956. Delayed fermentation of sucrose by certain haploid species of *Saccharomyces*. Antonie van Leeuwenhoek. 22 353-370.

Phaff, H.J., and J.W. Fell. 1970. *Cryptococcus* Kutzing emend. Phaff et Spencer, p. 1088-1145. In J. Lodder (ed.), The yeasts, a taxonomic study. North-Holland Publ. Co., Amsterdam.

Phaff, H.J., and J.F.T. Spencer. 1969. Improved parameters in the separation of species in the genera *Rhodotorula* and *Cryptococcus*, p. 59-65. In Proc. II Internat. Symp. on Yeasts, Bratislava.

Phaff, H.J., M.W. Miller, and M. Shifrine. 1956. The taxonomy of yeasts isolated from *Drosophila* in the Yosemite region of California. Antonie van Leeuwenhoek. 22: 145-161.

Phaff, H.J., E.M. Mrak, and O.B. Williams. 1952. Yeasts isolated from shrimp. Mycologia. 44: 431-451.

Poncet, S. 1967. A numerical classification of yeasts of the genus *Pichia* Hansen by a factor analysis method. Antonie van Leeuwenhoek. 33: 345-358.

Richards, M., and F.R. Elliot. 1966. Inhibition of yeast growth by streptomycin. Nature. 209: 536.

Roberts, C., and J.P. van der Walt. 1960. Investigations on maltose utilization in *Saccharomyces*. I. The acquisition of maltose fermentative ability by *S. capensis*. C.R. Trav. Lab. Carlsberg. 32: 19 - 34.

Ross, S.S., and E.O. Morris. 1962. Effect of sodium chloride on the growth of certain yeasts of marine origin. J. Sci. Food Agric. 13: 467-475.

Ross, S.S., and E.O. Morris. 1965. An investigation of the yeast flora of marine fish from Scottish coastal waters and a fishing ground off Iceland. J. Appl. Bacteriol. 28: 224 - 234.

Roth, F.J., Jr., D.G. Ahearn, J.W. Fell, S.P. Meyers and S.A. Meyer. 1962. Ecology and taxonomy of yeasts isolated from various marine substrates. Limnol. Oceanogr. 7: 178 - 185.

Ruinen, J. 1963. The phyllosphere. II Yeasts from the phyllosphere of tropical foliage. Antonie van Leeuwenhoek. 29: 425 - 438.

Saito, K. 1922. Untersuchungen uber die atmospharischen Pilzkeime. (German transl. from Japanese). Jpn. J. Bot. 1: 1 - 54.

Seshadri, R, and J.M. Sieburth. 1971. Cultural estimation of yeasts on seaweeds. Appl. Microbiol. 22: 507-512.

Schaeffer, A.B., and M. Fulton. 1933. A simplified method of staining endospores. Science. 77: 194.

Scheda, R., and D. Yarrow. 1966. The instability of physiological properties used as criteria in the taxonomy of yeasts. Arch. Mikrobiol. 55: 209-225.

Schultz, A.S., and S. Pomper. 1948. Amino acids as nitrogen sources for the growth of yeasts. Arch. Biochem. Biophys. 19: 184-192.

Shifrine, M., H.J. Phaff, and A.L. Demain. 1954. Determination of carbon assimilation patterns of yeasts by replica plating. J. Bacteriol. 68: 28-35.

Shinano, H. 1962. Studies on yeasts isolated from various areas of the North Pacific. Bull. Jpn. Soc. Sci. Fish. 28: 1113-1122.

Sieburth, J.M. 1959. Antibacterial activity of Antarctic marine phytoplankton. Limnol. Oceanogr. 4: 419-424.

Sieburth, J.M. 1960. Acrylic acid, an "antibiotic" principle in *Phaeocystis* blooms in Antarctic waters. Science. 132: 676-677.

Sieburth, J.M. 1961. Antibiotic properties of acrylic acid, a factor in the gastrointestinal antibiosis of polar marine animals. J. Bacteriol. 82: 72-79.

Sieburth, J.M., and A. Jensen. 1967. Effect of processing on the microflora of Norwegian seaweed meal, with observations on *Sporendonema minutum* (Høye) Frank and Iles. Appl. Microbiol. 15: 830-838.

Siepmann, R., and W. Hohnk. 1962. Uber Hefen und einige Pilze (Fungi imp., Hyphales) aus dem Nordatlanlik. Veroeff. Inst. Meeresforsch. Bremerhaven. 8: 79-98.

Sinclair, N.A., and J.L. Stokes. 1965. Obligately psychrophilic yeasts from the polar regions. Can. J. Microbiol. 11: 259-269.

Skinner, C.E. 1950. Generic name for imperfect yeasts, *Cryptococcus* or *Torulopsis*? Am. Midland Naturalist. 43: 242-259.

Smith, D.G., and R. Marchant. 1968. Chloramphenicol inhibition of *Pythium ultimum* and *Rhodotorula glutinis*. Arch. Mikrobiol. 60: 262-274.

Smith, D.G., and R. Marchant. 1969. Unbalanced cell-wall synthesis in chloramphenicol-grown *Rhodotorula glutinis*. Antonie van Leeuwenhoek. 35: 113-119.

Snow, J.E., and P.J. Beard. 1939. Studies on bacterial flora of north Pacific salmon. Food Res. 4: 563-585.

Soneda, M. 1961. On some yeasts from the Antarctic region. Jpn. Antarct. Res. Exped. Sci. Rep. Ser. E. 15, 10p.

Storck, R. 1966. Nucleotide composition of nucleic acids of fungi. II Deoxyribonucleic acids. J. Bacteriol. 91: 227-230.

Suehiro, S., and Y. Tomiyasu. 1962. Studies on marine yeasts. V Yeasts isolated from seaweeds. J. Fac. Agric. Kyushu Iniv. 12: 163-169.

Suehiro, S., Y. Tomiyasu, and O. Tanaka. 1962. Studies on marine yeasts. IV Yeasts isolated from marine plankton. J. Fac. Agric. Kyushu Iniv. 12: 155-161.

Taysi, I., and N. van Uden. 1964. Occurrence and population densities of yeast species in an estuarine-marine area. Limnol. Oceanogr. 9: 42-45.

Tsiklinsky, P. 1908. La flore microbienne dans les regions du Pole Sud. Expedition antarctique francaise (1903-1905), Paris. 117p.

Tsuchiya, T., Y. Fukazawa, and S. Kawakita. 1965. Significance of serological studies on yeasts. Mycopathol. Mycol. Appl. 26: 1-15.

Uden, N. van, and H. Buckley. 1970. *Candida* Berkhout, p. 893-1087. In J. Lodder (ed.), The yeasts, a taxonomic study. North-Holland Publ. Co., Amsterdam.

Uden, N. van, and L. do Carmo-Sousa. 1959. Further studies on the significance of temperature relations and vitamin deficiency patterns in yeast taxonomy, Port. Acta. Biol. Ser. B. 6: 239-256.

Uden, N. van; and R. Castelo-Branco. 1963. Distribution and population densities of yeast species in Pacific water, air, animals and kelp off southern California. Limnol. Oceanogr. 8: 323-328.

Uden, N. van, and M. Farinha. 1958. On the significance of temperature relations and vitamin deficiency patterns in the delimitation of yeast species. Port. Acta. Biol. Ser. B. 6: 161-178.

Uden, N. van, and J.W. Fell. 1968. Marine yeasts, p. 167-201. In M.R. Droop and E.J.F. Wood (eds.), Adv. in Microbiol. of the Sea, Vol. I. Academic Press, London.

Uden, N. van, and C.E. ZoBell. 1962. *Candida marina* nov. spec., *Torulopsis torrensii* nov. spec., and *T. maris* nov. spec., three yeasts from the Torres Strait. Antonie van Leeuwenhoek. 28: 275-283.

U.S. Hydrographic Service. 1960. Special Publication: Manual for Coding and Punching Oceanographic Data on Cards. Washington, D.C.

Vishniac, H.S., and G.A. Riley. 1961. Cobalamin and thiamine in Long Island Sound: patterns of distribution and ecological significance. Limnol. Oceanogr. 6: 36-41.

Volz, P.A., D.E. Jerger, A.J. Wurzburger, and J.L. Hiser. 1974. A preliminary survey of yeasts isolated from marine habitats at Abaco Island, the Bahamas. Mycopathol. Mycol. Appl. 54: 313-316.

Vuillemin, P. 1901. Les blastomycetes pathogenes. Rev. Gen. Sci. Pures Appl. 12: 732-751.

Walt, J.P. van der. 1962. Utilization of ethylamine by yeasts. Antonie van Leeuwenhoek. 28: 91-96.

Walt, J.P. van der, and A.E. van Kerken. 1961. Three new anascosporogenous yeasts. Antonie van Leeuwenhoek. 27: 206-212.

Walt, J.P. van der, and I.T. Tscheuschner. 1956. *Saccharomyces capensis*, nov. spec. A new yeast from South African grape must. Antonie van Leeuwenhoek. 22: 257-260.

Welch, A.M. 1962. Preliminary survey of fungistatic properties of marine algae. J. Bacteriol. 83: 97 - 99.

Whiffen, A.J. 1948. The production, assay and antibiotic activity of actidione, an antibiotic from *Streptomyces griseus*. J. Bacteriol. 56: 283 - 291.

Wickerham, L.J. 1946. A critical evaluation of the nitrogen assimilation tests commonly used in the classification of yeasts. J. Bacteriol. 52: 293 - 301.

Wickerham, L.J. 1951. Taxonomy of yeasts. U.S. Dept. of Agric. Bull. 1029, 56p.

Wickerham, L.J., and K.A. Burton. 1948. Carbon assimilation tests for the classification of yeasts. J. Bacteriol. 56: 363 - 371.

Wickerham, L.J., and K.A. Burton. 1954. A simple technique for obtaining mating types in heterothallic diploid yeasts, with special reference to their uses in the genus *Hansenula*. J. Bacteriol. 67: 303 - 308.

Wickerham, L.J., and M. Flickinger. 1946. Viability of yeasts preserved two years by the lyophil process. Brew. Dig. 21: 48 - 58.

Zerbe, W.B., amd C.B. Taylor. 1953. Sea water temperature and density reduction tables. U.S. Dept. of Commerce. Coast and Geodetic Survey. Special Publ. 298, Washington, D.C.

ZoBell, C.E. 1946. Marine microbiology. Chronica Botanica Co., Waltham, Massachusetts. 240p.

Zopf, W. 1889. Oxalsauregahrung (an Stelle von Alcoholgahrung) bei einen typischen (endosporen) Saccharomycetin (*S. hansenii* n. spec.).

APPENDIX I

SAMPLING DATA

TABLE 1

SAMPLING DEPTHS DURING THE SAMPLING PERIOD

Date	Water Depth (Meters)
1972	
Nov. 23	73.2
1973	
Jan. 25	64.0
Feb. 6	64.0
Mar. 21	69.5
May 15	71.3
May 29	73.2
June 13	69.5

TABLE 2

WATER TEMPERATURES DURING THE SAMPLING PERIOD

Date	Temperatures (^{o}C)		
	Surface	Middepth	Bottom
1972			
Nov. 23	3.0	3.0	3.0
1973			
Jan. 25	-1.0	-1.0	-1.0
Feb. 6	-1.0	-1.0	-1.0
Mar. 21	-1.0	-1.0	-1.0
May 15	0.5	-1.0	-1.0
May 29	0.5	-1.0	-1.0
June 13	3.5	-1.0	-1.5

TABLE 3

METEOROLOGICAL DATA DURING THE SAMPLING PERIOD

Date	Weather*	Air Temperature (°C)	Wind* Force	Direction
1972				
Nov. 23	Broken clouds	3.0	L B	W
1973				
Jan. 25	Overcast sky	3.0	L B	W
Feb. 6	Fair	-3.0	M B	NW
Mar. 21	Overcast sky.	1.5	F B	N
May 15	Overcast sky	8.0	M B	W
May 29	Overcast sky Rain	3.0	F B	SW
June 13	Overcast sky	6.5	L B	NNW

*Descriptions based on Manual for Coding and Punching Oceanographic Data on Cards (1960)

Weather:

Blue sky	clear or hazy atmosphere
Fair	few clouds; scattered sky
Mainly cloudy	1/2 sky overcast
Broken clouds	3/4 sky overcast
Overcast sky	whole sky overcast

Wind Force:

		Knots	Approx. m/sec.
C	calm	0-1	0.00- 0.52
L A	light air	1-3	0.52- 1.55
L B	light breeze	4-6	2.06- 3.09
G B	gentle breeze	7-10	3.61- 5.15
M B	moderate breeze	11-16	5.67- 8.24
F B	fresh breeze	17-21	8.76-10.82
S B	strong breeze	22-27	11.33-13.91
M G	moderate gale	28-33	14.42-17.00

TABLE 4

SEA CONDITIONS DURING THE SAMPLING PERIOD

Date	Sea State*	Sea Swell
1972		
Nov. 23	Smooth	Slight
1973		
Jan. 23	Smooth	Moderate
Feb. 6	Slight	Moderate
Mar. 21	Moderate	Moderate - Heavy
May 15	Smooth	Moderate
May 29	Slight - Moderate	Moderate - Heavy
June 13	Smooth	Heavy

*Descriptions based on Manual for Coding and Punching Oceanographic Data on Cards (1960)

Sea State:

	Height in m
Calm - glassy	0
Calm - rippled	0 -0.1
Smooth wavelet	0.1-0.5
Slight	0.5-1.2
Moderate	1.2-2.4
Rough	2.4-4.0
Very rough	4.0-6.1

TABLE 5

SALINITIES DURING THE SAMPLING PERIOD

Date	Salinities (parts/thousand)		
	Surface	Middepth	Bottom
1972			
Nov. 23	32.8	33.5	33.5
1973			
Jan. 25	30.3	35.7	36.3
Feb. 6	33.3	33.3	32.0
Mar. 21	34.0	34.0	34.0
May 15	31.2	33.8	35.4
May 29	31.5	34.1	36.7
June 13	34.6	34.6	38.5

APPENDIX II

FORMULARY

Synthetic Sea Water (Lyman and Fleming, 1940; modified by Moskovits and Flanagan, 1967)

NaCl	23.476g.
$MgCl_2.6H_2O$	10.629g.
Na_2SO_4 (anhydrous)	3.917g.
$CaCl_2.2H_2O$	1.459g.
KCl	0.664g.
$NaHCO_3$	0.192g.
KBr	0.096g.
Glass-distilled water	1000.000ml.

Add the salts directly to the distilled water. Dissolve each reagent completely before adding the next.

Medium "C" (Sieburth and Jensen, 1967; modified by Seshadri and Sieburth, 1971)

Yeast extract	1.0g.
Trypticase	10.0g.
Glucose	30.0g.
Agar	15.0g.
Synthetic sea water	1000.0ml.

After dissolving all components and before adding the agar, adjust the pH to 7.4 with 1 N NaOH. Following autoclaving at 121C for 15 minutes and reequilibration with the atmosphere, the pH of the medium is 7.0. When the medium is cooled to 45C, a sterile solution of chloramphenicol

at a concentration of 0.1g per liter is added to the medium.

Gram Stain

1. Ammonium Oxalate Crystal Violet (Hucker's)

Solution A

Crystal violet (90% dye content)	2.0g.
Ethyl alcohol (95%)	20.0ml.

Solution B

Ammonium oxalate	0.8g.
Distilled water	80.0ml.

Mix solutions A and B.

2. Iodine Solution

Iodine	1.0g.
KI	2.0g.
$NaHCO_3$	1.0g.
Distilled water	300.0ml.

3. Safranin Counterstain

Safranin O (2.5% in 95% ethanol)	10.0ml.
Distilled water	100.0ml.

Staining schedule

Stain for 1 minute with ammonium oxalate crystal violet. Pour off excess stain. Rinse in tap water and shake off excess water.

Immerse for 1 minute in iodine solution. Rinse with tap water and shake off excess.

Decolorize with 95% ethanol until no free stain washes off. Rinse with tap water and shake off excess.

Counterstain for 30 seconds with safranin. Rinse. Blot dry.

Yeast-Malt Extract (YM) Broth (Wickerham, 1951)

Yeast extract	3.0g.
Malt extract	3.0g.
Peptone	5.0g.
Glucose	10.0g.
Synthetic sea water	1000.0ml.

Dissolve all components. Adjust pH to 3.7 with dilute HCl. Autoclave for 15 minutes at 121C. Cool. Filter through Whatman No. 5 filter paper, doubled. Dispense in 5ml amounts into 20 x 125 mm screw-capped tubes. Autoclave for 15 minutes at 121C.

Yeast-Malt Extract (YM) Agar (Wickerham, 1951)

Yeast extract	3.0g.
Malt extract	3.0g.
Peptone	5.0g.
Glucose	10.0g.
Agar (Oxoid No. 3)	20.0g.
Synthetic sea water	1000.0ml.

After addition of all components and before adding the agar, the pH should be between 5 and 6. Autoclave for 15 minutes at 121C. Dispense in 10 ml amounts into 20 x 125

mm screw-capped tubes. Autoclave at 121C for 15 minutes. Cap tightly and slant.

Stock Vitamin Solution (van der Walt and van Kerken, 1961)

Inositol	0.2g.
Thiamine	0.1g.
Calcium pantothenate	0.04g.
Niacin	0.04g.
Pyridoxine hydrochloride	0.04g.
p-Aminobenzoic acid	0.02g.
Riboflavin	0.02g.
Folic acid	0.0002g.
Biotin	0.0002g.
Glass-distilled water	1000.0000ml.

Dissolve all components and sterilize by membrane filtration. Dispense in 10 ml aliquots into 20 x 125 mm screw-capped tubes and store at -10C.

For vitamin-enriched YM agar 0.1 ml of the solution is added aseptically to each tube, thoroughly mixed and the tubes slanted.

Freshwater Media

This media is made up by the same method used to make up YM broth and YM agar except that glass-distilled water is used instead of snythetic sea water.

2% Glucose-Yeast Extract-Peptone Water

Glucose	20.0g.

Peptone	10.0g.
Yeast extract	5.0g.
Synthetic sea water	1000.0ml.

Dissolve all components. Dispense in 5 ml amounts into 20 x 125 mm screw-capped tubes. Autoclave for 15 minutes at 121C.

2% Glucose-Yeast Extract-Peptone Agar

This is made by the same method as that used for 2% glucose-yeast extract-peptone water except for the addition of 20.0g. per liter of Oxoid agar No. 3. The medium is dispensed in 10 ml aliquots into 20 x 125 mm screw-capped tubes, autoclaved for 15 minutes at 121C and then the tubes are slanted.

Bacto-yeast Morphology Agar (Wickerham, 1951)

Bacto-yeast morphology agar (Difco)	35.0g.
Synthetic sea water	1000.0ml.

The composition of Bacto-yeast morphology agar is given in Lodder (1970). The medium is dissolved by gentle heating. It is autoclaved for 15 minutes at 121C and then dispensed aseptically into sterile Petri dishes.

Bacto-Yeast Nitrogen Base Medium (Wickerham and Burton, 1948)

Bacto-yeast nitrogen base (Difco)	6.7g.

Glass-distilled water	100.0ml.

The composition of Bacto-yeast nitrogen base is given in Lodder (1970). Bacto-yeast nitrogen base is dissolved, sterilized by membrane filtration and dispensed in 0.5 ml amounts into 13 x 100 mm sterile foam-plugged disposable tubes. The media is stored at 4.5 to 5C until used.

Carbon compounds for carbon assimilation tests

Each of the following compounds is dissolved in the given amounts in 90 ml of glass-distilled water, filter-sterilized, then added aseptically in 4.5 ml aliquots to tubes containing 0.5 ml of nitrogen basal medium. When not used immediately, each of the reagents is stored separately at 4.5 to 5C. When soluble starch or inulin is the carbon source, sterilization is carried out by autoclaving, instead of membrane filtration.

Maltose	0.500g.
i-Erythritol	0.509g.
Cellobiose	0.475g.
Raffinose	0.932g.
Sucrose	0.475g.
D-Mannitol	0.505g.
Inositol	0.500g.
Lactose	0.475g.
Melibiose	0.475g.
D-xylose	0.500g.
D-galactose	0.500g.
Melezitose	0.467g.
Ethanol	0.386g.
Inulin	0.450g.
L-arabinose	0.500g.
D-arabinose	0.500g.

Trehalose	0.475g.
L-Rhamnose	0.456g.
Glycerol	0.511g.
Soluble starch	0.426g.
L-Sorbose	0.500g.
Salicin	0.367g.
D-Glucitol	0.506g.
Galactitol	0.506g.
Ribitol	0.507g.
D-Ribose	0.500g.
D-Glucose	0.500g.
Glucono-δ-lactone	0.494g.
Methyl-α-D-glucoside	0.462g.
2-Keto-gluconic acid	0.539g.
Succinic acid	0.491g.
Citric acid	0.533g.
Lactic acid	0.500g.

The raffinose solution is made up at twice the concentration because the reaction may use only a part of the raffinose molecule.

Bacto-Yeast Carbon Base Medium (Wickerham, 1951)

Bacto-yeast carbon base (Difco)	11.7g.
Glass-distilled water	100.0ml.

The composition of Bacto-yeast carbon base is given in Lodder (1970). The medium is dissolved, then filter-sterilized. The solution is dispensed in 0.5 ml amounts into 13 x 100 mm sterile foam-plugged disposable tubes and stored at 4.5 to 5C until used.

Nitrogen compounds for nitrogen assimilation tests

Each of the following compounds is added in the given

amounts to 90 ml of glass-distilled water, filter-sterilized, then added in 4.5 ml aliquots to tubes containing the Bacto-yeast carbon base medium. When not used immediately, each of the reagents is stored separately at 4.5 to 5C.

Potassium nitrate	0.078g.
Ethylamine hydrochloride	0.064g.

Vitamin-Free Medium (Wickerham, 1951)

Bacto-vitamin-free medium base (Difco)	16.7g.
Glass-distilled water	100.0ml.

The composition of the Bacto-vitamin-free medium base is given in Lodder (1970). The medium is dissolved by gentle heating, filter-sterilized, then added in 0.5 ml aliquots to 13 x 100 mm foam-plugged tubes containing 4.5 ml of sterile distilled water. When not used immediately, the reagents are stored at 4.5 to 5C.

Cycloheximide Resistance Medium (Wickerham and Burton, 1948)

Cycloheximide (Sigma)	0.01g.
Glass-distilled water	90.0ml.

The cyloheximide is dissolved, the solution is filter-sterilized, then dispensed in 4.5 ml aliquots into 13 x 100 mm foam-plugged tubes. To this is added 0.5 ml of glucose assimilation medium, followed by thorough mixing.

Fermentation Basal Medium (Wickerham, 1951)

Yeast extract	4.5g.
Peptone	7.5g.
0.04% Bromthymol Blue	1.0ml. (Approximately)
Glass-distilled water	1000.0ml.

Bromthymol blue is made up according to the method described in Manual of Microbiological Methods (1957).

Yeast extract and peptone are dissolved. Bromthymol blue is added to give a deep green colour. From 0.9 to 1.1 ml of the indicator is required to give this colour. Two ml aliquots of the basal medium are dispensed into 13 x 100 mm foam-plugged tubes containing Durham tubes. The tubes are then autoclaved for 15 minutes at 121C. Upon cooling, the filter-sterilized carbohydrate solutions are added in 1 ml amounts. These solutions are made up at a concentration of 6% except in the case of raffinose which is made up at 12% concentration.

Yeast Infusion (Lodder and Kreger-van Rij, 1952)

Compressed yeast	200.0g.
Egg white from one egg	
Tap water	1000.0ml.

Dissolve components and autoclave for 15 minutes at 121C. Filter twice through double Whatman No. 5 filter paper.

APPENDIX III

DESCRIPTION OF SPECIES

Debaryomyces hansenii (Zopf) Lodder et Kreger-van Rij

There are eight species in the genus *Debaryomyces*. Fifty-nine strains have been examined. *Debaryomyces hansenii* (Zopf) Lodder et Kreger-van Rij has been indicated as the type species of the genus. Lodder (1970) includes thirty-six synonyms under this name. *D. hansenii* was first described by Zopf in 1889 and was named *Saccharomyces hansenii*. The genus, *Debaryomyces*, was established in 1909 by Kloecker and was characterized by its spherical, warty spores. Lodder and Kreger-van Rij (1952) felt that since this wartiness is not easily seen, other characteristics should become important in diagnosing the species. The following were the criteria decided upon: spherical or short-oval cells, pseudomycelium absent or rudimentary, vegetative cells haploid, conjugation between the mother cell and bud, spherical spores usually with a warty appearance, usually one spore per ascus (may have up to four per ascus), no or very weak fermentation, and no assimilation of nitrate.

Genus *Rhodotorula*

Harrison (as cited in Lodder, 1970) established the

genus; *Rhodotorula* in 1928 as an asporogenous yeast possessing red pigments. Lodder (1934) placed all asporogenous yeasts with carotenoid pigments (from red to yellow) under this genus.

There are nine species in the genus and *Rhodotorula glutinis* (Fresenius) Harrison is the type species. Diagnostic criteria of the genus include: spherical, ovoidal or elongated cells, multilateral budding reproduction, red and/or yellow carotenoid pigments, no fermentation, no assimilation of inositol, no production of extracellular polysaccharides, no gelatin liquefaction and no acid production on chalk agar.

Rhodotorula rubra (Demme) Lodder

Demme (as cited in Lodder, 1970) originally isolated this species in 1889 from milk and cheese and called it *Saccharomyces ruber*. His descriptions were based on the colour of the culture, cell morphology and the absence of fermentation. Vuillemin (1901) renamed the organism as a *Cryptococcus* species and Lodder (1934) transferred it to the genus, *Rhodotorula*. Lodder (1970) lists forty synonyms to *Rhodotorula rubra*. One hundred and eighty-one strains have been studied and these include strains that do not assimilate nitrate or lactose. They do

utilize maltose, sucrose, melezitose and raffinose. *R. rubra* is a very common species found in marine and terrestrial environments and also, partly due to its ability to grow at 37C, found in gastro-intentinal specimens from humans.

Rhodotorula glutinis (Fresenius) Harrison

Fresenius (1852) was the first to describe this species, isolating it from old starch paste and calling it *Cryptococcus glutinis*. Harrison (as cited in Lodder, 1970) in 1928, after studying the data left by Fresenius and also after examining several newly isolated species, felt that they should be included under the name, *Rhodotorula glutinis*. Lodder (1970) lists twenty-four synonymous under this name. Two varieties belong to this species: *R. glutinis* (Fresenius) Harrison var. *glutinis* and *R. glutinis* (Fresenius) Harrision var. *dairensis* Hasegawa et Banno. The latter requires thiamine for growth while the former is able to grow on vitamin-free medium. Also, *R. glutinis* var. *dairensis* utilizes nitrate and nitrite very weakly whereas *R. glutinis* var. *glutinis* shows a strongly positive reaction in such growth tests. Three hundred and seventy-one strains of the variety *glutinis* have been studied. *R. glutinis* is universal in distribution.

Rhodotorula graminis (di Menna)

This species was first described in 1958 when di Menna isolated it from pasture grass leaf surfaces in New Zealand (di Menna, 1958). Lodder (1970) lists two synonyms under this name. Sixty-nine strains have been studied. These are nitrate-positive and vitamin-independent. They do not assimilate melezitose, lactose or maltose.

Rhodotorula pallida (Lodder)

Lodder (1934) described this species after examining a strain labelled *Mycotorula muris*. There are no synonyms for this listed and only fifteen strains have been studied. It is a relatively rare species but has been obtained occasionally from marine sources (Ahearn, 1964).

Genus *Candida* (Berkhout)

Berkhout (1923) introduced the generic name *Candida* for asporogenous yeast-like fungi that were then in the genus *Monilia*.

There are eighty-one species in this genus and the type species is *Candida vulgaris* (Berkhout) which has been replaced by a synonym, *Candida tropicalis* (Castellani) Berkhout (Castellani, 1910). Diagnostic criteria of the genus include: globose, ovoid, cylindrical, elongate and sometimes irregularly shaped cells; reproduction is generally by

multipolar budding; presence of pseudomycelium; blastospores and chlamydospores may be formed but there are no ascospores, teliospores or ballistospores; extracellular polysaccharides may be produced; and alcoholic fermentation occasionally occurs.

The genus *Candida* appears to be closely related to the genus *Torulopsis* but they comprise a very heterogenous group of asporogenous yeasts. However, *Torulopsis* species lack the development of a pseudomycelium.

Candida diddensii (Phaff, Mrak et Williams) Fell et Meyer

Phaff, Mrak and Williams (1952) originally isolated this organism from shrimp collected in the Gulf of Mexico. They named it *Trichosporon diddensii*. Fell and Meyer (1967) transferred this species to the genus, *Candida* for they could find no arthrospores. Lodder (1970) lists seven synonyms under the name, *Candida diddensii*. Eleven strains have been studied. Two types of pseudomycelia are generally found; long, branching pseudohyphae with clavate tips and chains of shorter pseudohyphae bearing blastospores.

Candida diffluens (Ruinen)

Ruinen (1963) originally isolated this organism from

the surface of leaves of *Tillandsia usneoides* on trees in Dutch Guyana. There are no synonyms listed and only one strain has been studied. As in the genus, *Cryptococcus*, there are capsules present, urea is hydrolyzed and there is no fermentation but unlike *Cryptococcus* species, *Candida diffluens* does not assimilate inositol or produce starch-like extracellular polysaccharides.

Candida tropicalis (Castellani) Berkhout

Castellani (1910) first described this organism isolated from sputum samples in Ceylon. He named it *Oidium tropicale*. Berkhout introduced the name *Candida vulgaris* in 1923. This is the type species of the genus *Candida* and the name was transferred to *Candida tropicalis* by Diddens and Lodder (1942). There are fifty-seven synonyms for this name and thirteen strains have been thoroughly studied. Pseudomycelia are long and branched bearing blastospores. True mycelia may be formed.

Candida humicola (Daszewska) Diddens et Lodder

Daszewska (as cited in Lodder, 1970) originally isolated this species in 1912 from heath soil in Switzerland and named it *Torula humicola*. Diddens and Lodder (1942) placed this organism in the genus *Candida*. Lodder (1970) lists

seven synonyms under this name and twelve strains were studied. Pseudomycelia and true mycelia are abundantly formed. There is no fermentation. Starch formation is present.

Candida maritima (Siepmann) van Uden et Buckly nov. comb.

Siepmann (Siepmann and Höhnk, 1962) originally isolated this organism from water and shrimp eggs collected from the Northeastern Atlantic. He named this yeast *Trichosporon maritimum*. van Uden and Buckley (1970) transferred this to the genus *Candida* for, out of the three strains studied, they could find no arthrospores or mycelia. However, pseudomycelia and blastospores were present.

Genus *Cryptococcus* Kützing emend. Phaff et Spencer

According to Skinner (1950), Kützing originally isolated this genus in 1833 from a dirty window pane. He considered the organisms to be algae. However, the first recognizable species was *Cryptococcus hominis* isolated by Vuillemin (1901). This species is synonymous with *Cryptococcus neoformans*. He used the name *Cryptococcus* to describe asporogenous parasitic yeasts. At the time medical researchers tended to call the genus *Cryptococcus*, while non-medical researchers called it

Torulopsis; the latter being a fermenter and the former being a non-fermenter. Anderson (1917) was the first writer to use *Cryptococcus* to include both fermenting and non-fermenting species. Almeida (1939) gave up parasitic habitats as characteristic of *Cryptococcus*. Skinner (1950) proposed the separation of the genera *Cryptococcus* and *Torulopsis* on the basis of the absence of fermentation in *Cryptococcus* species. Lodder and Kreger-van Rij (1952) added to this distinction the ability of *Cryptococcus* species to form capsules and produce starch-like compounds.

There has also been confusion between species of the genera *Rhodotorula* and *Cryptococcus*. Starch production was accepted as diagnostic of the latter and carotenoid pigment synthesizing ability was considered characteristic of the former. There are exceptions to these criteria in both genera. Therefore, Phaff and Spencer (1969) proposed a new diagnostic criterion, the ability to assimilate inositol in the genus *Cryptococcus*. This removed from *Rhodotorula* all species that could produce starch.

There are seventeen species in the genus *Cryptococcus* and some of the diagnostic criteria are: spheroidal, amoeboid or polymorphic cells; reproduction by multilateral budding; most strains form capsules and produce starch or starch-like compounds; carotenoid pigments may be formed;

no pseudomycelia or mycelia formed; no fermentation; and all species assimilate inositol.

Cryptococcus albidus (Saito) Skinner

There are three varieties to this species: variation *albidus*, variation *aerius* (Saito) Phaff et Fell nov. var., variation *diffluens* (Zach) Phaff et Fell nov. var.

Cryptococcus albidus var. *albidus*

This variety differs from var. *aerius* in that it is positive for starch production. Variation *albidus* differs from var. *diffluens* in that it assimilates lactose, D-galactose and methyl-α-D-glucoside.

Saito (1922) originally isolated this yeast from the air in Tokyo and called it *Torula albida*. Skinner (1950) changed the name to *Cryptococcus albidus* observing that no fermentation took place.

There are fourteen synonyms to this variety and two hundred and thirty-six strains were studied. *Cr. albidus* var. *albidus* is universal in distribution and found frequently in both marine and terrestrial habitats.

Cryptococcus infirmo-miniatus (Okunuki) Phaff et Fell

nov. comb.

Okunuki (1931) originally isolated this organism from the air in Tokyo and called it *Torula infirmo-miniata*. Hasegawa and Banno (1964) renamed it for its pink colour and lack of assimilation of lactose as described by Lodder (1934) but mainly for its production of extracellular starch, low optimum temperature (20C) and its requirement for biotin. Ahearn and Roth (1966) observed pigmented chlamydospore-like cells and hyphal elements. These resembled teliospores and Phaff and Fell (1970) felt that this may lead to reclassification into the basidiomycetous yeasts.

There are three synonyms for *Cr. infirmo-miniatus* as listed by Lodder (1970) and twenty-three strains were studied. Isolations have been made from a variety of sources including marine and terrestrial habitats.

Genus *Leucosporidium* (Fell, Statzell, Hunter et Phaff)

This is one of the yeast-like genera found in the order, Ustilaginales.

The genus *Leucosporidium* may present organisms in different phases. Firstly, there is the haploid phase where the cells are oval to elongate and budding. The culture is white to cream-coloured and mucoid. The

second possibility is the dikaryotic phase where there has been conjugation between opposite mating types. There are clamp connections at each septum and the mycelium develops terminal or intercalary teliospores. The third phase consists of the self-sporulating yeast phase. The cells are oval to elongate and budding. There are no clamp connections but a pseudomycelium may be present. There are large intercalary or terminal teliospores.

Fell, Statzell, Hunter and Phaff (1969) used the term, *Leucosporidium*, to describe *Candida*-like organisms, with an asexual stage unlike that found in *Rhodotorula* and similarities to *Rhodosporidium*.

There are seven species in this genus and the type species is *Leucosporidium scottii*, isolated by Fell et al. (1969) from Antarctic marine waters. There are two synonyms for this name and Diddens and Lodder (1942) originally described one as *Candida scottii*. This was transferred to *Leucosporidium* when Fell et al. (1969) found the perfect form.