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A biosystematic investigation of medically important yeasts

Nancy Christiansen Ballot
University of the Pacific

A BIOSYSTEMATIC INVESTIGATION OF MEDICALLY IMPORTANT YEASTS

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Nancy Christiansen Ballot

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Nancy Christiansen Ballot

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Department Chairman or Dean:

F. R. Hunter

Thesis Committee:

Chairman

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INTRODUCTION AND HISTORICAL REVIEW

Since early times, the action of yeasts has been known and used by man in the production of ethanol from various carbohydrate sources. Not until the late 19th century did Schwann and Cagnard-LaTour determine that the sediment in the bottom of the fermentation vessels was a living organism. The name yeast is probably derived from the Dutch word gist or the German word gischt for foam, a characteristic of the fermentation process.

Originally bacterial methods utilizing chiefly fermentation studies were used in the classification of yeasts. More recently, additional tests have been added, and today the general procedures include: growth at 37°C, pellicle formation in Sabouraud broth, the production of hyphae, pseudohyphae, chlamydospores and germ tubes, capsule formation, assimilation and fermentation tests involving 12 and five carbohydrates, respectively, urease activity, nitrate utilization, resistance or sensitivity to cycloheximide, and occasionally production of pigment on birdseed agar (Table I).

Growth at 37°C is not specifically tested for since clinical specimens are always cultured routinely at 35-37°C, and in the case of fungal cultures, both at 35-37°C

and room temperature, 20-25°C.

Pellicle formation is usually performed after a presumptive identification has been made, and is rarely resorted to as a routine procedure today.

Hyphae can be defined as the branched cells produced by fungal colonies which may or may not be septate. Each hypha has a uniform width with growth occurring at the distal hyphal tip producing elongation of these tip cells. This is in contrast with pseudohyphae which are produced by budding, giving rise to smaller cells at the distal portion of the filament which are not of uniform width.

The ability of certain yeasts and particularly Candida albicans to produce chlamydospores was first reported by Benham (1931) using corn meal agar (CMA). Soon after, it became evident that not all strains of C. albicans produce chlamydospores on this medium. Other media were developed and characterized in an effort to find a more reliable one.

Reid et al. (1953) suggested that chlamydospore production depends on zein, a protein and chief ingredient of corn meal. Because of technical difficulties involved in preparation of the medium and differences in content of this basic protein in white and yellow corn meals, production of chlamydospores is inconsistent. Nickerson and Mankowski (1953) suggested that the production of chlamydospores is due to the absence of reducing sugars and recommended

the use of purified starch as a medium. Liu and Newton (1955) suggested that chlamydospore production results from growth under unfavorable conditions such as anaerobic atmosphere, alkaline pH of 8.2 and deficiency in nutrients, and these conditions are met by their phosphate-buffered thioglycollate medium. They were of the opinion that starch agar of Nickerson and Mankowski could maintain these three conditions whereas corn meal agar does not because of a lowered pH which encourages production of pseudomycelia rather than chlamydospores. The addition of Tween 80 to rice infusion agar was reported by Taschdjian (1957) to improve the production of chlamydospores. Kelly and Funigiello (1959) reported on a comparative study using corn meal agar, corn meal agar with Tween 80, and rice infusion agar with Tween 80. All 18 strains of C. albicans used in this study produced chlamydospores in 24 hours but the addition of Tween 80 to corn meal and rice infusion agar stimulated production of chlamydospores in larger numbers. More recently Behesti et al. (1975) introduced a medium, rice-infusion-oxgall-Tween 80 (RIOT) agar for the production of both chlamydospores and germ tubes.

The production of germ tubes in serum has been suggested by Taschdjian et al. (1960) as a rapid method for the presumptive identification of C. albicans. Johnson (1954) reported that Candida stellatoidea as well as C. albicans produce germ tubes. Mackenzie (1965) objected to the use of the term "germ tube" on the grounds that it

does not refer to the germination of fungal spores and suggested its replacement by "pseudogerm tube". While Mackenzie is technically correct most investigators continue the usage of the more familiar term.

Capsule formation is a characteristic of most species of the genus Cryptococcus. In clinical work the technique is almost limited to the detection of Cryptococcus neoformans in cerebrospinal fluid.

Assimilation of a carbon source by yeasts is dependent upon availability of enzymes necessary to utilize that particular compound for energy needs and growth. Two basic methods, each with several modifications have been in use for many years: the auxanographic plate method of Beijerinck introduced in 1889 and Wickerham's enriched broth procedure (Wickerham and Burton, 1948). Beijerinck introduced the auxanogram procedure which became a useful tool in yeast taxonomy. Beijerinck's method called for a dry carbohydrate to be sprinkled over a small area of a nutrient agar in a Petri dish which had been previously seeded with specific yeast. Assimilation is evident in the form of growth around the "sprinkles". In modified forms (Drouhet and Couteau's method as reported in Schnall, 1967; DiMenna, 1955; Adams and Cooper, 1974; Huppert et al., 1975; Land et al., 1975) this auxanogram method is still used.

Wickerham and Burton (1948), in an effort to improve methods used in classification of yeasts, investigated 70

carbon sources using 100 strains representing 22 genera of yeasts in a vitamin enriched chemically defined medium, Yeast Nitrogen Base (YNB)[1], to determine the most useful carbon compounds for yeast classification. The addition of vitamins to the medium permitted growth of many species previously unable to utilize the medium. The investigators found 22 of the 70 compounds tested to be useful for taxonomic work on the basis of consistent results. Wickerham's method differed from Beijerinck's in that the yeast was introduced into a carbohydrate broth and assimilation determined by turbidity. Wickerham's broth tube method was modified by Martin and Schneidau (1970) through the addition of agar and by Adams and Cooper (1974) through the addition of agar and pH indicator. Another modification (Hall et al., 1972) utilized the semi-solid oxidation fermentation (OF) medium of Elizabeth O. King.

In the classification of medically important yeasts, the twelve sugars used are glucose, maltose, sucrose, lactose, galactose, melibiose, cellobiose, inositol, xylose, raffinose, trehalose, and dulcitol.

In contrast with assimilation which may occur under an anaerobic or aerobic condition, fermentation of a carbohydrate is always anaerobic usually resulting in the production of organic acids and/or alcohols and gas.

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[1]Difco Laboratories, Detroit, Michigan.

In 1921, Stelling and Dekker developed a method utilizing Einhorn fermentation flasks to indicate CO_2 production from different carbohydrates as a means of characterizing yeasts. In 1949, Bouthilet, Neilson, Mrak and Phaff found Durham tubes as CO_2 collecting vessels to be as reliable as Einhorn flasks. Lodder (1967), however, favored the use of Einhorn flasks over Durham tubes for the collection of CO_2. This is the bacterial method for the study of fermentation. However, instead of 24-48 hours incubation for bacteria, 10-15 days are needed to obtain definitive results with yeasts. This is due to incubation at lower temperature (25°C) and a slower generation time (20-24 hours) for yeasts compared with 20-30 minutes for most mesophilic bacteria. Rapid methods were consequently introduced where a small amount of medium, a large inoculum, and 37°C incubation are used. With such a system, fermentation is observed in one to five days. Harper (1975) and Huppert et al. (1975) devised one such rapid fermentation method using yeast suspension and Key Fermentation Tablets[1] in plastic tubes which are overlaid with a vaspar plug (1:1 Vaseline:paraffin). The production of gas is usually evident in 24 hours through displacement of the vaspar plug. The five sugars usually used in these fermentation studies are glucose, maltose, sucrose, lactose, and galactose.

The ability of yeasts to utilize nitrate as the

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[1]Key Scientific Products, Los Angeles, California.

sole source of nitrogen was a criterion Stelling and Dekker used in 1931 to distinguish between the genera Pichia and Hansenula. Other nitrogen sources: ammonium sulfate, urea, asparagine and peptone, were investigated by Lodder in 1934 using the auxanogram method developed by Beijerinck in 1889. This method was applied by Langeron and Guerra in 1938 to the study of Candida. Wickerham (1946) developed a synthetic medium, Yeast Carbon Base (Difco), enriched with trace elements (boric acid, copper sulfate, potassium iodide, ferric chloride, manganese sulfate, sodium molybdate, and zinc sulfate) and vitamins (biotin, calcium pantothenate, folic acid, inositol, niacin, p-aminobenzoic acid, pyridoxine, riboflavin, and thiamine). He found that most yeasts could utilize many nitrogen sources when the medium is supplied with these vitamins and trace elements. The nitrate assimilation test used today utilizes KNO_3 in Wickerham's broth.

Cycloheximide, an antibiotic fungicide, produced by Streptomyces griseus has been shown to inhibit many saprophytic fungi and some pathogenic species (Phillips and Hanel, 1950; Fuentes et al., 1952; Georg, 1953; and Georg et al., 1954). Resistance or susceptibility to cycloheximide is therefore one more criterion for the identification of medically important yeasts (Silva-Hutner and Cooper, 1974).

Another test, introduced by Weld (1952), and not included in Table I, but recommended for the presumptive identification of C. albicans is the production of feathery

or spidery colonies on Levine eosin methylene blue (EMB) incubated at 37°C and 10% CO_2 atmosphere. Weld (1952) described "spidery" colonies as "irregular masses of clear, oval, cells, from which radiate one or more filaments of mycelium of varying length tapering off to bare ends. Along filaments are found small clusters of spores. Many of the filaments are seen to penetrate deep into the agar." This is in contrast with the "feathery" colonies where the "central mass of cells is smaller than that of a spidery colony. It appears as a dark mass of furry brown in which the individual cells are indistinct. Each colony is entirely encircled by a fringe of long filaments bearing small spore clusters, the branches tapering off to long bare ends." C. stellatoidea will develop mycelia on this medium consisting "of a loose network of scattered spores with long intertwining tubes without the formation of colonies."

Recently, Analytab Products, Inc. (API)[1] introduced a kit, API 20 C for identification of medically important yeasts. Each API 20 C strip system consists of microtubes containing dehydrated substrates for fermentation and assimilation reactions which are reconstituted when a pure yeast suspension is added to each microtube. The strips are incubated and read after 24-72 hours. This system had just been introduced into the American market at the time

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[1]API, 200 Express Street., Plainview, New York, 11803.

that this study was begun. The only evaluation of this system that this author has been able to find is by Miller and Lu (1976) who compared it with conventional standard media. These investigators found disagreement in biochemical reactions between the two systems although "the final identifications agreed in 100% of the cases and were based on their complete reaction pattern." Their study was based on 44 strains belonging to six species of yeasts.

Yeast infections in hospitals today involve basically two groups of patients: 1. those on prolonged broad-spectrum antibiotic therapy; and 2. those who are immunologically-compromised (patients on corticosteroids, immunosuppressive drugs, or irradiation therapy). In most instances, no attempt is made to accurately identify the yeast. Often a few tests are performed to determine if the yeast isolate is "Candida albicans" or "saprophytic yeasts".

The purpose of this study is twofold: 1. conduct an epidemiological survey of yeasts found in clinical material; and 2. suggest an identification scheme that would identify yeasts of medical importance in the shortest possible time.

MATERIALS AND METHODS

Between January and December, 1976, 128 strains of yeasts were isolated from routine (100) and fungal (28) cultures at the Microbiology Department of Dameron Hospital Laboratory, Stockton, California. The isolates were obtained from the following sites (Table II): vaginal tract (60), respiratory tract (48), urinary tract (15), eye (2), and one each from ear, nail and feces. Each strain was subcultured on a brain heart infusion (BHI) (Difco) agar plate and incubated at 37°C for 48 hours. An isolated colony was selected from each plate, transferred to a Sabouraud agar slant, and stored at 4°C for further processing.

The suspension used to inoculate the various media was a light suspension diluted in sterile distilled water and read against a Wickerham card (three India ink lines each about 0.75 mm wide on a white card). A light inoculum is that dilution in which the lines observed through a 16 mm diameter test tube can be seen as distinct bands.

The germ tube test and EMB plates (for spidery colonies) were incubated at 37°C, fermentation tests at 30°C, and all the others at 25°C. Incubation time varied from three hours for the germ tubes to 14 days for carbohydrate

assimilation studies. Incubation temperature (in degrees Celsius) and time are listed under each test.

1. Growth at 37°

This was determined when a streaked BHI plate was incubated at 37° and also when the germ tube test was conducted.

2. Hyphae-pseudohyphae

The production of hyphae or pseudohyphae was determined by streaking a portion of a colony from a Sabouraud agar plate on corn meal agar plate with Tween 80 (CMT80)[1] using Dalmau's method (laying a sterile coverglass over the streaks), incubated at room temperature for 24-48 hours. Growth was examined under low dry objective (100X).

3. Chlamydospore production

The production of chlamydospores was also determined on CMT80 by examining growth at 450X magnification for presence of round, thick-walled highly refractile cells.

4. Germ tube production

One-half milliliter of human serum was inoculated directly with a portion of an isolated colony from Sabouraud agar slant and incubated at 37° for three hours. Then a drop of serum was placed on a slide and examined under low dry objective for the production of germ tubes, parallel-

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[1]Microbiological Media, Concord, California.

walled extrusions from the mother cell without any constriction at the point of origin.

5. Production of feathery-spidery colonies

A small amount of a light suspension of a three-day growth of yeast was streaked on Levine's EMB (Difco), and incubated in a CO_2 jar at 37°. After 24-48 hours incubation the colonies were examined under 100X magnification for the typical cellular morphology of feathery or spidery colonies.

6. Carbohydrate assimilation (Wickerham broth tube method)

Each carbohydrate was prepared as a 10% solution in 6.7% yeast nitrogen base (YNB, Difco) and filter-sterilized using a 0.22u Millipore filter[1]. Half ml aliquots were added to 4.5 ml sterile water in 16x150 mm screw top tubes. Each tube was inoculated with 0.1 ml aliquots of dilute yeast suspension and incubated at 25° for 14 days. The tubes were shaken vigorously and observed visually for turbidity on days 1, 2, 4, 7, 10 and 14 following inoculation.

7. Carbohydrate fermentation (modified method developed by the author)

Fermentation studies were performed on 1% solutions of each of five sugars (glucose, maltose, sucrose, lactose and galactose) in an agar medium. Twenty ml of 10% filter-

[1]Millipore Corporation, Medford, Massachusetts.

sterilized (0.22u Millipore filter) sugar solution was added to 180 ml of previously autoclaved (15 lbs. psi for 15 minutes) phenol red agar base (Difco). While still melted, 1.5 ml aliquots were dispensed in sterile 10x75 mm capped tubes. The media in the tubes were stored at room temperature until ready to be used.

Each sugar was inoculated with 0.1 ml light suspension of yeast, and stabbed several times with a sterile needle to insure penetration of the yeast into the agar. The medium was then overlaid with approximately 0.5 ml melted vaspar, incubated at 30^{o} and observed daily for seven days. Fermentation was determined by gas production which raised the vaspar plug above the surface of the agar. If no gas was evident, the production of acid as evidenced by a change from red to yellow was noted.

8. Nitrate assimilation

Eleven and two-tenth grams of yeast carbon base (YCB, Difco) were dissolved in 100 ml distilled water, filter-sterilized (0.22u Millipore filter) and pipetted as 2 ml aliquots into 15x100 mm Petri dishes. To each Petri dish 1/10 ml light suspension and 10 ml sterile 2% agar were added, mixed by swirling and allowed to harden.

To the outer edge of one half of the plate approximately one mg KNO_3 crystals was added and to the outer edge of the other half one mg peptone. Plates were incubated at 25^{o} for three days. Nitrate assimilation is indicated by growth around both the peptone and the nitrate

crystals. Growth around the peptone only is considered a negative test for nitrate assimilation.

9. Urease production

Christensen urea agar (Microbiological Media) was inoculated with a small portion of a 48 hour culture grown on Sabouraud agar using an inoculating needle, and incubated at 25° for five days. A change in color to cerise indicates a positive test.

10. Cycloheximide resistance

Mycosel agar (Microbiological Media) was inoculated with a small portion of a 48-hour culture grown on Sabouraud agar slant, and incubated at 25° for five days. Growth of colonies indicates resistance to cycloheximide.

11. Statistical methods

Ninety-five percent confidence intervals (Dixon and Massey, 1969) were set about the sample probability that a carbohydrate would be assimilated or fermented within 48 hours for three species, C. albicans, C. tropicalis and Torulopsis glabrata. Confidence intervals were not set for the other seven species due to small sample size.

Chi square analysis was performed on Burroughs B6700 computer at University of Pacific using the SPSS (Nie et al., 1970) program CROSSTAB. This test was used to determine the occurrence of species-dependent reactions in the assimilations of xylose and trehalose on day one.

RESULTS

The 128 yeast isolates belong to 10 species in three genera (Table II): Candida (110), Torulopsis (17), Trichosporon (1). The genus Candida is represented by seven species, C. albicans being the most common with 97 strains or 75.8% of the total, C. tropicalis (6), C. stellatoidea (2), C. guillermondii (1), C. krusei (1), C. parasilosis (1), and Candida sp. (2). The genus Torulopsis is represented by two species T. glabrata (16) and T. pintolopesii (1), and the genus Trichosporon by a single isolate Tr. pullulans.

The two Candida strains reported as Candida sp. remain unidentified. Assimilation tests suggest either C. albicans, C. tropicalis or C. parapsilosis. By fermentation studies the two strains do not fit into any of these three species. They differ from C. albicans by their failure to ferment maltose, produce germ tubes, spidery colonies or chlamydospores. Furthermore, they are sensitive to cycloheximide. From C. tropicalis, they differ in their inability to ferment maltose, and from C. parapsilosis by their ability to ferment sucrose. The two strains which clearly belong in the genus Candida gave identical results in their physical and biochemical reactions including the

production of unusual lavender pigmented colonies on EMB. One of the two strains was isolated from an eye culture of one patient, the other from the sputum of another.

Table II lists the distribution of the isolates by clinical material. For C. albicans the most common site is the vaginal tract (46 strains) followed by the respiratory tract (42) and urinary tract (5). T. glabrata is represented by nine strains in the vaginal tract, five in the urinary tract, and two in the respiratory tract. Three strains of C. tropicalis were isolated from the urinary, two from the respiratory, and one from the vaginal tracts.

1. Growth at 37°

All 128 strains of yeasts showed growth at 37°.

2. Hyphae-pseudohyphae

All species of Candida produced pseudohyphae and the one strain of Trichosporon pullulans produced hyphae on CMT80 in three days but none of the 17 species of Torulopsis did.

3. Chlamydospore production

Chlamydospores were produced by 90 of 97 strains of C. albicans. Five strains produced chlamydospores in three days, 86 strains by six days, and 90 strains (92.8%) by day nine. Seven strains of C. albicans did not produce any chlamydospores after 14 days of incubation.

No C. stellatoidea or C. tropicalis strains produced any chlamydospores in this study.

4. Germ tube production

Germ tubes were produced by 95 strains (97.7%) of C. albicans and by the two strains of C. stellatoidea.

5. Production of feathery-spidery colonies

On EMB, typical feathery or spidery colonies of C. albicans were produced in 95 strains (97.9%). One strain of C. stellatoidea produced colonies resembling feathery colonies but were thread-like with few blastospores. No other species produced the typical colonies.

6. Carbohydrate assimilation

Assimilation tests were performed, observed and recorded on days one, two, four, seven, ten, and 14. Table III lists the cumulative results of assimilation of carbohydrates. The 97 strains of C. albicans assimilated six carbohydrates: glucose, maltose, sucrose, galactose, xylose, and trehalose. The days at which assimilations were recorded were as follows: glucose by 96 strains on day one, by the one remaining strain on day two; maltose by 60 strains on day one, 36 on day two, and the one remaining strain by day four; sucrose by 42 strains on day one, 53 on day two and the remaining two by day four; galactose by 25 strains on day one, 70 strains on day two and the remaining two strains by day four; xylose by one strain on day one, 71 strains on day two, and the remaining

25 strains by day 14; trehalose by one strain on day one, 80 strains on day two and the remaining 16 strains by day seven.

The 95% confidence intervals for the assimilation of carbohydrates within 48 hours by C. albicans are in Table IV.

Six strains of C. tropicalis assimilated the seven carbohydrates: glucose, maltose, sucrose, galactose, xylose, trehalose, and cellobiose. The days at which assimilations were recorded were as follows: glucose and maltose by six strains on day one; sucrose by five strains on day one and the one remaining strain on day two; galactose by four strains on day one, one on day two, and the one remaining strain by day four; xylose by four strains on day one, and the two remaining strains on day two; trehalose by five strains on day one and the one remaining strain on day two; cellobiose by one strain on day two and the five remaining strains by day seven.

The 95% confidence intervals for the assimilation of carbohydrates within 48 hours by C. tropicalis are in Table IV.

One strain of C. parapsilosis assimilated six carbohydrates: glucose, maltose, sucrose, galactose, xylose and trehalose. The days at which assimilations were recorded were as follows: glucose on day one, maltose, sucrose, galactose, xylose and trehalose on day two.

Two strains of C. stellatoidea assimilated five

carbohydrates: glucose, maltose, galactose, xylose and trehalose. The days at which assimilations were recorded were as follows: glucose by two strains on day one; maltose by one on day one and by the one remaining on day two; galactose by two strains on day two; xylose by two strains by day four; trehalose by one strain by day two and the one remaining by day four.

One strain of C. guillermondii assimilated ten carbohydrates: glucose, maltose, sucrose, galactose, melibiose, cellobiose, xylose, raffinose, trehalose, and dulcitol. The days at which assimilations were recorded were as follows: glucose, maltose, sucrose, galactose, cellobiose, and raffinose on day one; xylose on day two; melibiose, trehalose, and dulcitol on day four.

One strain on C. krusei which characteristically assimilates only glucose did so on day one.

Two strains of Candida sp. assimilated six carbohydrates, glucose, maltose, sucrose, galactose, xylose, and trehalose follows: glucose and galactose by both strains on day one; maltose and xylose by one on day one and the second on day two; sucrose by two strains on day two; trehalose by one strain on day two and the second by day 14.

Sixteen strains of T. glabrata assimilated two carbohydrates, glucose and trehalose as follows: glucose by 14 strains on day one and two remaining strains by day ten; trehalose by 11 strains on day one, two on day two; the three remaining strains by day 14.

The 95% confidence intervals for the assimilation of glucose and trehalose within 48 hours by T. glabrata are in Table IV.

One strain of T. pintolopesii which characteristically assimilates only glucose did so on day one.

One strain of Tr. pullulans assimilated all the carbohydrates with the exception of dulcitol in the following manner: glucose, maltose, galactose, melibiose, cellobiose, inositol, xylose, raffinose, and trehalose on day one; sucrose and lactose on day two.

On the basis of 707 sugars assimilated, involving all carbohydrates and all yeasts encountered in this study, 643 (91%) assimilations occurred within 48 hours.

7. Carbohydrate fermentation

Fermentation tests were performed on all 128 strains of yeasts utilizing glucose, maltose, sucrose, lactose, and galactose. Tests were observed daily for seven days with results recorded on days one, two, and four. Table V lists the cumulative results for the carbohydrate fermentation indicating production of acid (A) or acid and gas (AG).

The 97 strains of C. albicans fermented four carbohydrates, glucose, maltose, sucrose, galactose producing acid and gas or acid. With glucose, acid and gas were produced by 61 strains on day one, 22 strains on day two and three strains on day four*; while acid was produced by eight

*Acid alone was proudced on day one or two, gas appeared on day four.

strains on day one and six strains on day two. With maltose, acid and gas were produced by 55 strains on day one, 22 strains on day two and four strains on day four*; while acid was produced by 10 strains on day one and ten on day two. With sucrose, acid and gas were produced by seven strains on day two and 12 strains* by day four; acid was produced by six strains on day one, 77 strains on day two and seven strains by day four. With galactose, acid and gas were produced by five strains on day four*; acid was produced by 37 strains on day one, and by 60 strains on day two.

The 95% confidence interval is at the 0.93 ± 0.005 level for fermentation of sucrose within 48 hours as evidenced by acid or acid and gas. The 95% confidence intervals for the fermentations of glucose, maltose and galactose are at the 1.00 ± 0 level which indicates completion of reaction within 48 hours.

The six strains of C. tropicalis fermented the four carbohydrates glucose, maltose, sucrose and galactose producing acid and gas or acid. With glucose, acid and gas were produced by three strains on day one and two strains on day two; acid was produced by one strain on day one. With maltose, acid and gas were produced by one strain on day one and four strains on day two; acid was produced by one strain on day two. With sucrose, acid and gas were produced

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*Acid alone was produced on day one or two, gas appeared on day four.

by three strains on day one and three strains on day two. With galactose, acid was produced by two strains on day one, four strains on day two. By day four, three strains had produced gas.*

The 95% confidence intervals for the fermentation of glucose, maltose, sucrose and galactose are at the 1.00 ± 0 level.

The one strain of C. parapsilosis fermented glucose and galactose with production of acid by day two.

The two strains of C. stellatoidea fermented glucose, maltose and galactose. Glucose and maltose were fermented with production of acid and gas by day one. Galactose was fermented with production of acid by one strain on day one and one strain on day two.

The single strain of C. guillermondii fermented glucose, sucrose, and galactose as follows: acid and gas were produced from glucose on day one, sucrose on day two; acid alone was produced from galactose on day two.

The one strain of C. krusei fermented glucose with the production of acid and gas on day two.

The two strains of Candida sp. fermented glucose, sucrose and galactose. With glucose both strains produced acid and gas on day two. With sucrose two strains produced acid on day two, one strain produced acid and gas by day four.* With galactose, both strains produced acid on day two.

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*Acid alone was produced on day one or two, gas appeared on day four.

Sixteen strains of T. glabrata fermented only glucose as follows: acid and gas were produced by nine strains on day one, five strains by day two; acid was produced by one strain on day one and one strain on day two.

The 95% confidence intervals for the fermentation of glucose is at the 1.00 ± 0 level.

The single strain of T. pintolopesii fermented only glucose with production of acid and gas on day one.

Tr. pullulans single strain fermented all five carbohydrates with production of acid as follows: glucose on day one, and maltose, sucrose, lactose, and galactose on day two.

8. Nitrate assimilation

The assimilation of KNO_3 as the sole source of nitrogen was characteristic of Tr. pullulans only.

9. Urease production

Production of urease was detected only in the single strain of Tr. pullulans.

10. Cycloheximide resistance

All 97 strains of C. albicans were resistant to cycloheximide as were C. stellatoidea, C. tropicalis, C. guillermondii, and Tr. pullulans.

The results on all physical and biochemical tests are summarized in Table VI.

DISCUSSION

This study was undertaken in part as an epidemiological survey of yeasts isolated at Dameron Hospital, Stockton, California, between January and December, 1976. One hundred twenty-eight strains of yeasts of human origin representing ten species were isolated and identified. The most common species was C. albicans representing 97 strains or 75.8%, followed by T. glabrata 16 strains or 12.5%, and C. tropicalis six strains or 4.7%. These three species represented 93% of all the isolates.

Other investigators (Bump and Kunz, 1968; Dolan, 1971) have reported the same three species as the most commonly isolated yeasts. In a survey involving 1443 isolates, at Massachusetts General Hospital, Boston, Massachusetts, Bump and Kunz (1968) reported a prevalence of 43.2% C. albicans, 14.6% C. tropicalis, and 15.6% T. glabrata for a total of 73.4%. Other yeasts found were: C. krusei 2.4%, C. parapsilosis 2.3%, miscellaneous Candida species 11.3%, various Torulopsis species 2.4%, Rhodotorula sp. 1.0%, Cryptococcus neoformans 0.6%, Cryptococcus sp. 0.4%, Geotrichum-like species (including Trichosporon sp.) 1.0%, and 5.3% were not identifed.

At Mayo Clinic, Rochester, Minnesota, Dolan's (1971) study involved 530 strains of yeasts of which C. albicans represented 28.3%, C. tropicalis 21.7% and T. glabrata 34.6% for an overall total of 84.6%. In all three surveys, representing different geographical regions of the United States, C. albicans, C. tropicalis and T. glabrata were the most common yeasts although not in the same order of prevalence.

This difference in incidence of the three species may be partially explained on the basis of isolation site. In this study 96.1% were isolated from three sites (Table III): vaginal tract 60 of 128 (46.9%), respiratory tract 48 of 128 (37.5%), and urinary 15 of 128 (11.7%). Bump and Kunz (1968) reported 45.6% from respiratory, 27.5% urinary, and 6.0% vaginal. Similarly Dolan (1971) reported 38.9%respiratory, 24.5% urinary and 12.1% vaginal. The higher incidence in the vaginal tract from Dameron Hospital may be explained by the fact that the majority of cases were from Emergency Room out-patients whereas, the majority of respiratory and urinary tract isolates were from in-patients.

Identification of clinically important yeasts has traditionally concentrated on determining the "pathogen" C. albicans from "non-pathogenic" yeasts. This is based primarily on four tests (germ tube formation, resistance to cycloheximide, formation of feathery or spidery colonies on Levin EMB and production of chlamydospores) which allow

rapid identification. C. albicans is identified on the basis of a positive reaction in any two of these four tests.

Comparing the results obtained in this study with the records at Dameron Hospital indicated that the hospital microbiologists identified correctly 83 of the 97 strains (85.6%) as C. albicans. Five strains identified by the microbiologists as C. albicans proved to be T. glabrata (3), Tr. pullulans (1), and C. stellatoidea (1). Those isolates that were not identified as C. albicans were reported as "saprophytic yeasts" or "Candida sp." The detailed testing in this investigation placed them in the following species: T. galabrath (13), T. pintolopesii (1), C. albicans (14), C. tropicalis (6), C. krusei (1), C. guillermondii (1), C. stellatoidea (1), C. parapsilosis (1), Candida sp. (2).

It is evident from these results that although 85.6% of C. albicans may be correctly identified by these four tests, a few strains belonging to this species may not be. Furthermore, non-C. albicans strains cannot be speciated by these tests. Biochemical tests and growth characteristics are used as adjunct or supplemental procedures for further speciation. The problem, however, is one of obtaining results in the shortest possible time, within 48-72 hours, and using the minimum number of tests possible.

The traditional methods of carbohydrate assimilation and fermentation requiring up to 14-day incubation

are the most reliable ones. In this study, the carbohydrate assimilation method of Wickerham and Burton (1948) was chosen for its accepted accuracy. Results were recorded at frequent intervals to determine whether identification in 48 hours was possible. The sample sizes of the three species: C. albicans, C. tropicalis and T. glabrata were sufficient to be statistically analyzed (Table IV). It is evident from this study that most assimilations do occur within 48 hours. Of 707 positive sugar assimilation tests by all strains of yeasts, 91% occurred within this period. Therefore, in most cases the test would be reliable in the necessarily limited time.

Whereas the assimilation method is reliable, the number of carbohydrates to be tested for each identification makes the procedure very cumbersome. In addition, several species have similar assimilation patterns e.g. C. albicans, C. parapsilosis and some strains of C. tropicalis, or C. krusei and T. pintolopesii which would require further testing for speciation.

Most strains of C. albicans would have assimilated the six sugars characteristic of the species within 48 hours. C. tropicalis would assimilate the same six sugars within 48 hours and cellobiose after 48 hours. The rates of assimilation of xylose and trehalose at 24 hours differed significantly ($\alpha = 0.005$) and therefore can be used to distinguish between C. albicans or C. tropicalis.

The introduction of a modified method for fermentation of sugars is designed to obtain rapid results by

utilizing a minimum quantity of test sugar with a relatively large inoculum. Harper (1975) introduced a rapid method for fermentation utilizing the fermentation tablets (Key Scientific Products) for glucose, maltose, and sucrose, incubating at 37° and observing results in 24 hours. Although her method yields results comparable to conventional method (Segal and Ajello, 1976) in a shorter period of time (24 hours versus 14 days) her methods omitted lactose and galactose. The present method utilized small aliquots (1.5 ml) of all five sugars in 1% concentration in phenol red agar base and a vaspar plug. Incubation at 30° rather than 37° is preferred for more uniform results. The production of acid as well as acid and gas was noted although Hall et al. (1972) and Silva-Hutner and Cooper (1974) have emphasized that fermentation in yeast studies compared with bacterial testing implies the production of gas and not acid alone. As noted by Wickerham (1951) and Lodder (1967) gas may be produced but the collection vessel may not accurately reflect this. Therefore, it was felt justifiable to record both the production of acid as well as gas.

Fermentation by the modified rapid method was observed for seven days but changes occurring after four days were in the form of color reversal back to red due to change in metabolic products after available carbohydrate was exhausted, and an apparent loss of gas with the vaspar plug returning to original position.

The production of acid from the fermented carbohydrate was observed in all cases within 48 hours with the exception of seven strains of C. albicans which did not produce acid from sucrose until day four. Some strains later produced gas as noted in Table V.

Because of small sample size further studies are necessary on all species, with the possible exception of C. albicans, before drawing any conclusions as to reliability, speed, and accuracy using this modified method. Regardless of these current limitations, the method is certainly one which could be incorporated into the identification routine of a clinical laboratory on the basis of rapidity of results, and limited space requirements due to small size of the test tubes used in this procedure.

Tables V and VI indicate that C. albicans and C. tropicalis fermented the same sugars but the rate and reaction (acid or acid-gas) of sucrose fermentation appeared to be different. Most strains of C. albicans (83/97) produced acid but no gas by day two (only 7/97 produced acid and gas on day two). This is in contrast with the six strains of C. tropicalis of which three produced acid and gas on day one, and the others by day two.

Chlamydospore formation is a characteristic of C. albicans, C. stellatoidea and certain strains of C. tropicalis (Table I). The production of chlamydospores in this study occurred only in 90 strains (92.8%) of C. albicans using Dalmau method on CMT80. After three-day

incubation at 25° chlamydospores were evident in only five strains (5.2%). While certain investigators (Gordon et al., 1952; Reid et al., 1953; Liu and Newton, 1955; Kelley and Funigiello, 1959; Haley and Standard, 1973; and Webb et al., 1975) recommend up to three-day incubation, this study suggests that a longer incubation period is necessary. While only 5.2% produced chlamydospores within three days, 87.6% produced them in four to nine days and seven strains produced none. None of the strains of C. stellatoidea or C. tropicalis produced chlamydospores.

Although the production of chlamydospores is an identifying feature of C. albicans, the limitations due to the time needed for production, make this test less useful when rapid identification is necessary.

The production of hyphae-pseudohyphae on CMT80 is characteristic of Candida and Trichosporon but not Torulopsis. Since these structures are observable within 48 hours the test should be included in routine procedures. It is evident then that the usefulness of CMT80, when results are desired within a 48 hour period, is as a medium for the production of hyphae and pseudohyphae rather than chlamydospores.

Germ tube formation is a characteristic of C. albicans and C. stellatoidea (Table I). In this study 95 strains of C. albicans (97.9%) and two strains of C. stellatoidea (100%) produced germ tubes in three hours. Taschdjian et al. (1960) reported that three of 39 strains

(7.7%) of C. albicans did not produce germ tubes. Dolan (1971) and Haley and Standard (1973) reported production of germ tubes in 85% and 98%, respectively by their strains of C. albicans.

Webb et al. (1973) and Huppert et al. (1975) suggested microscopic examination for germ tubes within three hours after inoculation of the serum. If incubation is extended beyond that time, C. tropicalis may form pseudohyphae which superficially resemble germ tubes. The six strains of C. tropicalis examined in this study all formed pseudohyphae at three hours. The two structures can be distinguished by examining the proximal area of the tube for constriction which occurs only in pseudohyphae.

Germ tube formation is the most rapid test available for presumptive identification of C. albicans and should certainly be a part of any identification schema. It is easily performed and requires minimal preparations of media, or equipment.

Cycloheximide resistance is a characteristic of C. albicans, C. stellatoidea, C. pseudotropicalis, C. guillermondii, Rhodotorula rubra, Trichosporon beigelii, Tr. capitatum, Tr. pullulans, and Tr. penicillatum (Table I). In this study, resistance to cycloheximide was observed in all strains of C. albicans, C. stellatoidea, C. tropicalis, C. parapsilosis, C. guillermondii, and Tr. pullulans (Table VII).

The results involving the six strains of C. tropicalis

are in disagreement with those reported in the literature (Silva-Hutner and Cooper, 1974). Roberts et al. (1976) reported growth on cycloheximide-containing media with two strains of C. tropicalis. The tests for cycloheximide resistance involving the six strains in this study were repeated and the same results obtained.

The test for cycloheximide resistance is easily performed and results are obtained within 24-48 hours.

Weld (1952) described the typical feathery or spidery colonies formed on Levine EMB in 10% CO_2 at 37°. In this study 95 strains (97.9%) of C. albicans produced the typical colonies on this medium. One strain of C. stellatoidea produced a thread-like growth which superficially resembles feathery-spidery colonies of C. albicans. This type of growth was described by Weld (1952) for C. stellatoidea growing on Levine EMB in the presence of CO_2 unlike C. albicans which only forms typical growth in the presence of CO_2.

Levine EMB is readily available in any clinical microbiology laboratory because it is used in most routine cultures, and the results are observable within 24-48 hours.

Unrelated to formation of feathery-spidery colonies but because growth was on EMB plate it was noted that three of the six strains of C. tropicalis produced "fried egg" colonies characterized by raised dark purple centers surrounded by lavender borders. Further investigation is needed to verify if this feature could be a reliable characteristic.

One objective of this study was to recommend a schema for rapid and accurate identification of medically important yeasts. In most laboratories four tests, germ tube, chlamydospore, feathery colonies, and resistance to cycloheximide, are commonly used to distinguish C. albicans from "saprophytic yeasts". Confirmation of C. albicans and identification of these "saprophytic yeasts" requires the use of assimilation and fermentation studies. Assimilation studies are more reliable than fermentation reactions. If the API 20 C system alluded to in the introduction proves to be as reliable as the limited studies of Miller and Lu (1976) indicate, then a major problem in yeast identification is solved. The system is also expensive and time consuming with an average cost of about seven dollars per test including labor. Furthermore, in the opinion of this author, the germ tube test should be performed because it permits rapid presumptive identification of C. albicans. Reaction to cycloheximide is part of the API 20 C system but according to Miller and Lu (1976) the results may not be dependable. According to the present study all ten species of yeasts encountered can be identified in 48 hours by fermentation tests involving five sugars, the germ tube test in serum, type of growth on Levin EMB and CMT80 and reaction to cycloheximide (Table VII). On the basis of these tests identification of the three most common yeast species, C. albicans, C. tropicalis, and T. glabrata can be made with a high degree of reliability; however,

larger sample sizes are needed for C. stellatoidea, C. parapsilosis, C. krusei, C. guillermondii, T. pintolopesii, and Tr. pullulans because the fermentation method is a modification of existing methods and the reactions may not be as reliably characteristic as they appear with the present limited sample size. There does appear to be a unique fermentation pattern for C. stellatoidea, C. parapsilosis, and Tr. pullulans. Although, C. albicans and C. tropicalis ferment the same four carbohydrates, the production of gas within 48 hours from sucrose appears to be unique to C. tropicalis. The reactions of the commonly used media are additional criteria. C. guillermondii could be differentiated from Candida sp. by reaction on cycloheximide. C. krusei could be differentiated from the genus Torulopsis by production of hyphae on CMT80. Growth at 42^{o} distinguishes T. pintolopesii from T. glabrata.

SUMMARY

One hundred and twenty-eight strains of yeast representing ten species in three genera: Candida (C. albicans 97, C. tropicalis 6, C. stellatoidea 2, C. guillermondii 1, C. krusei 1, C. parapsilosis 1, Candida sp. 2), Torulopsis (T. galabrata 16, T. pintolopesii 1), and Trichosporon (Tr. pullulans 1) were isolated from clinical material. They were studied with respect to their biochemical and physical properties.

A schema is presented for rapid and accurate identification of the ten species encountered in this study. These tests include serum for formation of germ tubes, EMB for feathery or spidery colonies, fermentation of carbohydrates (glucose, maltose, sucrose, lactose, and galactose), CMT80 for production of pseudohyphae and chlamydospores, and Mycosel for resistance to cycloheximide.

Other yeast species are encountered occasionally that would not fit into the above schema. The more detailed procedures would then be necessary.

TABLE I

Cultural and biochemical characteristics of yeasts frequently isolated from clinical specimens

Species	Growth at 37 C	Pellicle, broth	Pseudo/true hyphae	Chlamydospores	Germ tubes	Capsule, India ink	Assimilations												Fermentations					Urease activity	KNO_3 utilization	Brown colonies on birdseed agar	Cycloheximide resistance
							Glucose	Maltose	Sucrose	Lactose	Galactose	Melibiose	Cellobiose	Inositol	Xylose	Raffinose	Trehalose	Dulcitol	Glucose	Maltose	Sucrose	Lactose	Galactose				
Candida albicans	+	−	+	+	+	−	+	+	+	−	+	−	−	−	+	−	+	−	F	F	−*	−	F	−	−	−	+
C. stellatoidea	+	−	+	+	+	−	+	+	−	−	+	−	−	−	+	−	+*	−	F	F	−	−	−	−	−	−	+
C. tropicalis	+	+	+	+[b]	−	−	+	+	+	−	+	−	+*	−	+	−	+	−	F	F	F	−	F	−	−	−	−
C. parapsilosis	+	−	+	−	−	−	+	+	+	−	+	−	−	−	+	−	+	−	F	−	−	−	F*	−	−	−	−
C. krusei	+	+	+	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	F	−	−	−	−	+*	−	−	−
C. pseudotropicalis	+	−	+	−	−	−	+	−	+	+	+	−	+	−	+*	+	−	−	F	−	F	F	F	−	−	−	+
C. guillermondii	+	−	+	−	−	−	+	+	+	−	+	+	+	−	+	+	+	+	F	−	F	−	F	−	−	−	+
C. rugosa	−	−	+	−	−	−	+	−	−	−	−	+	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−
Cryptococcus neoformans	+	−	*R*	−	−	+	+	+	+	−	+	−	+	+	+	+*	+	+	−	−	−	−	−	+	−	+	−
C. albidus var. *albidus*	−*	−	+*	−	−	+	+	+	+	+*	−*	−*	+	+	+	+	+*	+	−	−	−	−	−	+	+	−	−
C. albidus var. *diffluens*	+	−	−	−	−	+	+	+	+	−	−*	+*	+	+	+	+	+	+*	−	−	−	−	−	+	+	−	−
C. luteolus	−	−	−	−	−	−	+	+	+	−	+	+	+	+	+	−*	+	+*	−	−	−	−	−	+	−	−	−
C. laurentii	+	−	−	−	−	+	+	+	+	+	+	+*	+	+	+	+*	+	+	−	−	−	−	−	+	−	−	−
C. uniguttulatus	−	−	−	−	−	+	+	+	+	−	−*	−	−*	+	+	+*	+*	−	−	−	−	−	−	+	−	−	−
C. terreus	+	−	−	−	−	+	+	+*	−*	−*	+*	−	+	+	+	−	+	+*	−	−	−	−	−	+	+	−	−
C. gastricus	−	−	−	−	−	+	+	+	−*	−	+	−	+	+	+	−	+	−	−	−	−	−	−	+	−	−	−
Rhodotorula glutinis	−	−	−	−	−	−	+	+	+	−	+*	−	+	−	+	+	+	−	−	−	−	−	−	+	+	−	−
R. rubra (*mucilaginosa*)	+*	−	−	−	−	−	+	+	+	−	+*	−	+*	−	+	+	+	−	−	−	−	−	−	+	−	−	+*
Saccharomyces cerevisiae	+	−	+*	−	−	−	+	+	+	−	+	−	−	−	−	+	+*	−	F	F	F	−	F	−	−	−	−
Torulopsis glabrata	+	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	+	−	F	−	−	−	−	−	−	−	−
T. pintolopesii	+[c]	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	F	−	−	−	−	−	−	−	−
Trichosporon beigelii	+*	+	+	−	−	−	+	+*	+*	+	+	+*	+	+*	+	+*	+*	+*	−	−	−	−	−	+*	−	−	+
T. capitatum	+	+	+	−	−	−	+	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+
T. pullulans	+	+	+	−	−	−	+	+	+	+	+	+*	+	+*	+	+*	+	−	−	−	−	−	−	+	+	−	+
T. penicillatum	+	+	+	−	−	−	+	−	−	−	+	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	+
T. inkin	+	+	+	−	−	−	+	+	+	+	+	−	+	+	+	−	+	−	−	−	−	−	−	−	−	−	−
Geotrichum candidum	−	+	+	−	−	−	+	−	−	−	+	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−

[a] Asterisks indicate strain variation; R, rare. Under Assimilations, plus signs indicate growth greater than the control; under Fermentations, F indicates that sugar is fermented (i.e., gas is produced).

[b] Occasional strains of *C. tropicalis* produce tear drop-shaped chlamydospores.

[c] *T. pintolopesii* is a thermophilic yeast capable of growth at 40 to 42 C.

Photocopied from Silva-Hutner and Cooper (1974).

TABLE II

Frequency of Site of Isolation

Species	Total	Site of Isolation						
		Vaginal	Respiratory	Urinary	Eye	Ear	Nail	Feces
Candida albicans	97	46	42	5	1	1	1	1
C. tropicalis	6	1	2	3				
C. stellatoidea	2	2						
C. guillermondii	1	1						
C. krusei	1			1				
C. parapsilosis	1			1				
Candida sp.	2		1		1			
Torulopsis glabrata	16	9	2	5				
T. pintolopesii	1	1						
Trichosporon pullulans	1		1					
Total	128	60	48	15	2	1	1	1

TABLE III

Number of Strains Assimilating Carbohydrates

Carbohydrate	A. Candida albicans						B. Candida tropicalis						C. Candida parapsilosis					
	Days of Assimilation						Days of Assimilation						Days of Assimilation					
	1	2	4	7	10	14	1	2	4	7	10	14	1	2	4	7	10	14
Glucose	96	1					6						1					
Maltose	60	36	1				6							1				
Sucrose	42	53	2				5	1						1				
Lactose																		
Galactose	25	70	2				4	1	1					1				
Melibiose																		
Cellobiose								1	4	1								
Inositol																		
Xylose	1	71	22	2		1	4	2						1				
Raffinose																		
Trehalose	1	80	12	3	1		5	1						1				
Duicitol																		

TABLE III (cont.)

Carbohydrate	D. *Candida stellatoidea*						E. *Candida guillermondii*						F. *Candida krusei*						G. *Candida* sp.					
	Days of Assimilation						Days of Assimilation						Days of Assimilation						Days of Assimilation					
	1	2	4	7	10	10	1	2	4	7	10	14	1	2	4	7	10	14	1	2	4	7	10	14
Glucose	2						1						1						2					
Maltose	1	1					1												1	1				
Sucrose							1													2				
Lactose																								
Galactose		2					1												2					
Melibiose									1															
Cellobiose							1																	
Inositol																								
Xylose			2					1											1	1				
Raffinose							1																	
Trehalose		1	1						1											1				1
Dulcitol									1															

TABLE III (cont.)

Carbohydrate	H. Torulopsis glabrata						I. Torulopsis pintolopesii						J. Trichosporon pullulans					
	Days of Assimilation						Days of Assimilation						Days of Assimilation					
	1	2	4	7	10	14	1	2	4	7	10	14	1	2	4	7	10	14
Glucose	14			1	1		1						1					
Maltose													1					
Sucrose														1				
Lactose														1				
Galactose													1					
Melibiose													1					
Cellobiose													1					
Inositol													1					
Xylose													1					
Raffinose													1					
Trehalose	11	2	1	1		1							1					
Dulcitol																		

TABLE IV

95% Confidence Intervals for Probability of Carbohydrate Assimilation within 48 Hours

Carbohydrate	*C. albicans* (n=97)	*C. tropicalis* (n=6)	*T. glabrata* (n=16)
Glucose	1.00 ± 0	1.00 ± 0	0.88 ± 0.17
Maltose	0.99 ± 0.02	1.00 ± 0	
Sucrose	0.98 ± 0.03	1.00 ± 0	
Galactose	0.98 ± 0.03	0.83 ± 0.30	
Xylose	0.74 ± 0.09	1.00 ± 0	
Trehalose	0.84 ± 0.07	1.00 ± 0	
Cellobiose		0.17 ± 0.30	

TABLE V

Number of Strains Fermenting Carbohydrates

Carbohydrate		A. *Candida albicans*			B. *Candida tropicalis*			C. *Candida parapsilosis*			D. *Candida stellatoidea*		
		Day 1	Day 2	Day 4	Day 1	Day 2	Day 4	Day 1	Day 2	Day 4	Day 1	Day 2	Day 4
Glucose	AG*	61	22	3***	3	2					2		
	A**	8	6		1				1				
Maltose	AG	55	22	4***	1	4					2		
	A	10	10			1							
Sucrose	AG		7	12***	3	3							
	A	6	77	7									
Lactose	AG												
	A												
Galactose	AG			5***			3***						
	A	37	60		2	4			1		1	1	

*AG indicates both acid and gas produced

**A indicates acid but no gas produced

*** acid alone was produced on days 1 and 2, gas appeared on day 4.

TABLE V (cont.)

Carbohydrate		E. Candida guillermondii			F. Candida krusei			G. Candida sp.		
		Day 1	Day 2	Day 4	Day 1	Day 2	Day 4	Day 1	Day 2	Day 4
Glucose	AG	1			1				2	
	A									
Maltose	AG									
	A									
Sucrose	AG		1							1***
	A								2	
Lactose	AG									
	A									
Galactose	AG									
	A		1						2	

***acid alone was produced on days 1 and 2, gas appeared on day 4.

TABLE V (cont.)

Carbohydrate		H. Torulopsis glabrata			I. Torulopsis pintolopesii			J. Trichosporon pullulans		
		Day 1	Day 2	Day 4	Day 1	Day 2	Day 4	Day 1	Day 2	Day 4
Glucose	AG	9	5		1					
	A	1	1					1		
Maltose	AG									
	A								1	
Sucrose	AG									
	A								1	
Lactose	AG									
	A								1	
Galactose	AG									
	A								1	

TABLE VI

Summary of Physical and Biochemical Tests

Species	Number of strains	Assimilation Within 14 Days												Fermentation Within 48 Hours																						Hyphae-pseudohyphae	Chlamydospores	Germ tube	Nitrate assimilation	Urease activity	Feathery colonies	Growth at 37°	Cycloheximide resistance
		Glucose	Maltose	Sucrose	Lactose	Galactose	Melibiose	Cellobiose	Inositol	Xylose	Raffinose	Trehalose	Dulcitol	Glucose		Maltose		Sucrose		Lactose		Galactose																					
														AG	A	AG	A	AG	A	AG	A	AG	A																				
Candida albicans	97	+	+	+		+				+		+		83	14	77	20	7	83				97													+	90	95			95	+	97
C. tropicalis	6	+	+	+		+		+		+		+		5	1	5	1	6					6													+						+	6
C. parapsilosis	1	+	+	+		+				+		+			1								1													+						+	
C. stellatoidea	2	+	+			+				+		+		2		2							2													+		2				+	2
C. guillermondii	1	+	+	+		+	+	+		+	+	+	+	1				1					1													+						+	1
C. krusei	1	+												1																						+						+	
Candida sp.	2	+	+	+		+				+		+		2					2				2													+						+	
Torulopsis glabrata	16	+										+		14	2																					-						+	
T. pintolopesii	1	+												1																						-						+	
Trichosporon pullulans	1	+	+	+	+	+	+	+	+	+	+	+			1		1		1		1		1													+			+	+		+	1

\+ positive test or growth
A Acid production
AG Acid and gas

TABLE VII

Tests for 48 Hour Identification for Medically Important Yeasts

Species	Germ tube	Feathery-spidery colonies	Fermentation					Hyphae/chlamydospores CMT80		Cycloheximide Resistance	Additional tests
			glucose	maltose	sucrose	lactose	galactose*				
Candida albicans	+	+	AG/A**	AG/A	A	-	A	+	+	R	
C. tropicalis	-	-	AG/A	AG/A	AG	-	A	+	-	R***	
C. parapsilosis	-	-	A	-	-	-	A	+	-		
C. stellatoidea	+	-	AG	AG	-	-	A	+	-	R	
C. guillermondii	-	-	AG	-	AG	-	A	+	-	R	
C. krusei	-	-	AG	-	-	-	-	+	-	-	
Torulopsis glabrata	-	-	AG/A	-	-	-	-	-	-	-	Growth 42C -
T. pintolopesii	-	-	AG	-	-	-	-	-	-	-	Growth 42C +
Trichosporon pullulans	-	-	A*	A*	A*	A*	A	+	-	R	

* in this study, acid was produced but no gas

** AG = acid and gas; A = acid but no gas

*** in this study, all strains were resistant

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