Chapter 3

## RESULTS

According to the mprphollogical and physiological study, 319 tested strains were identifled as) candida albicans 142 ( $44.24 \%$ ), the highest incidence, C. Eropiealis=51 (15.80\%), C. parapsilosis 8 (2.49\%), Torulopsis glabrece $18(5.61 \%)$. The less frequent species of Candida included the following, C. pseudotropicalis, C. krusei, C. guilliermondii, C. 2ambica arata. catenulata (see Table 2). The mamber of organism in the genes Crygtogocous, only Cr. neoformans 51 (15.89\%) was isolated from himar and amimenk goureas (see Table 2). For the genus Trichosporon, there weri $27(8,41 \%$ ) isolates of Tr. cutaneum and one of Tr . pullulans, Jo the last genus of isolated yeasts, Rhodotorula, R Dubra (2), R. graminis (9), goglutinis (1) were found in this research see Table 2). And the last 2 isolated strains were undifferentiated to species even based on morphological and
 glutineous efice tween or agar, these two isolates were identified as candigat? R specimens in this study. (see Table 2 ).

Classical and rapid carbohydrates assimilation.

For the comparative study of conventional and rapid assimilaticn of 15 kinds of carbohydrate it was found that Torulopsis and Candida krusei have correct result there were no false positive and

Table 2. Identification of Tested Strains


Table 2. Identification of Tested Strains (cont.)

flase negative as shown in table 4.1 and table 4.4. Table 4.2 was the result of C. albicans, the data showed that some kind of carbohydrates were not concomitant results. These carbohydrates were sorbose ( $4.14 \%$ different), cellobiose ( $100 \%$ different), soluble starch (1.38\% different) and arabinose ( $93,20 \%$ bjefferent). By using chi-squaretest of these four different valuestaten it was found that only rapid assimilation of cellobiose and ardibinose were significantly different from conventional method. 9 fropicailis, table 4.3, only cellobiose was $7.55 \%$ different by ohg chi-squaretest at $p=0.01$ it was found that rapid cellobiose assimilation was not significant different from conventional assignation. As, from table 4.4 , results of $c$. parapsilosis, it showed thar apia assimilation of trehalose, xylose, arabinose were significanfil different from conventional assimilation at $\mathrm{p}=0.01$. Compared result 5 . was found that rapid assimilation of galactose? trehalose, melibiose, xylose and inositol were significantly different from conventional assimilation at $p=0.01$.

## ศนย์วิทยทรัพยากร

For II. cutaneum, the result was shown in table 4.7, that only
 conventional assimilation. Other carbohydrates also had different results on these two methods but no significance by using $p=0.01$. From table 4.8, result of R. graminis, the data had shown that rapid assimilation of sorbose and xylose had significant differences from conventional assimilation.

The table 4.1 showed results of uncommon isolated species and rapid assimilation was different from conventional assimilation in some kind of carbohydrates, one of interesting carbohydrate was dulcitol which has been assimilated by $C$. quilliermondii by conventional assimilation |hyt/ kould not assimilated by rapici assimilation. The other uncomme, isorated species c. Catenulata; its data was not shown, द्याso had dfficiont result between rapid and classical methods on sofff/kinds of carbohydrate such as xylose lactose, raffinose and insftof for Tr. pullulans; galactose for R. glutinis, cellobjose, thehalose, ratfinosc and xylose for C . utilis: trehalose and rafingse for fifllusitamees maltose for Cr. albidus; sucrose, maltose ofliobiósictrahalose, lactose, melibiose, raffinose, and soluble starch for Gostrichuyd candidum; trehalose, soluble starch and arabinose for R. mingtay

Carbohydrates fermentation
 Rhodotorula, Trorulopsis in medically important yeasts Candida and
 in the genus Candidawere able to ferment glucose, c. albicans 40 ( $28.17 \%$ ) isolates could ferment galactose, 50 (39.43\%) isolates could ferment trehalose, all isolates could ferment maltose, but could not ferment sucrose and lactose. All of c. tropicalis ( 51 isolates) were able to ferment sucrose, 2 of 51 isolates could not ferment galactose and maltose and 3 of 51 isolates could not ferment trehalose. The same as $\mathbf{C}$. albicans, all isolates of C. tropicalis could not ferment

Table 3. : Characteristic of standard strain

Species
INK GT MS TEM URE $K$ Ch PI A Film


| ATCC $=$ | American Type Culture Collection |
| ---: | :--- |
| CDC $=$ | Division of Mycotic Disease, |
|  | Center for Disease Control, Atlanta |
| NIH $=$ | National Institute of health, Bethesda, Maryland |
| TIST $=$ | Thailand Institute of Scientific |
|  | and Technology Research |
| DUKE U. = Duke University |  |

Table 3.1 : Characteristic of standard strain (sugar fermentation)

|  |  | FD | FG | FS | FM | FT | FL |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 1 Candida albicans | ATCC 10231 | + | + | - | + | + | - |
| 2 c . albicans | CDC 85-00000xyz | $+$ | $+$ | - | + | + | - |
| 3 c. tropicalis | ape 354985258 | $+$ | + | + | + | + | - |
| C. parapsilosis | 0065085 | + | $+$ | - | - | - | - |
| c. guilliermondii | C3 $85-031998$ | + | + | + | - | + | - |
| 6 c. krusei | DC $85-031997$ | + | - | + | - | - | - |
| $7 \text { C. stellatoides }$ |  | + | - | - | - | + | - |
| 8 Geotric |  |  | - | - | - | - | - |
| 9 |  |  | + | - | + | + | - |
| $10$ |  |  | + | + | + | + | - |
| 11 c. pseudotropic |  |  | + | $+$ | - | + | - |
| 12 c. parapsilosis |  | + | + | - | - | - | - |
| 13 T. glabrata |  | + | - | - | - | + | - |
|  | 159 5133 |  | - | - | - | - | - |
| 15 c .1 lusitaniae | S72/515 |  | - | $+$ | - | + | - |
| 16 c . utilis | TIST 5146 |  | $+$ | - | - | - | - |
| 17 Pichia quilleermondii | TIST 5142 |  | $+$ | + | - | $+$ | - |
| 18 T. glabrata | TIST 514 |  | - | - | - | + | - |
| 19 Rhodotorula rubra | TIST 5124 |  | - | - | - | - | - |
|  |  |  |  | - | - | - | - |
|  |  |  |  | - | - | - | - |
| 22 cr . laurentii L (KE U. A |  |  |  |  | - | - | - |
|  |  |  |  |  | - | - | - |
| 24 Trichosporon beijelli | DUKE U. | - | - | - | - | - | - |


| $\mathrm{FD}=$ GLUCOSE FERMENTATION | $\mathrm{FM}=$ MALIOSE FERMENTATION |
| :--- | :--- |
| FG $=$ GALACIOSE FERMENTATION | $\mathrm{FT}=$ TREHALOSE FERMENTATICN |
| FS $=$ SUCROSE FERMENTATION | FL $=$ LACIOSE FERMENTATION |

Table 3.2 : Characteristic of standard strain (Sugar assimilation)





Table 4.3 : Results of , classical and rapid carbohydrate assimilation tests for Torulposis glabrata Total $=20$ strains (two standard strain included)


$$
\begin{aligned}
\% \text { DIEF } & =\% \text { Difference } \\
\text { ND } \quad & =\text { Not done }
\end{aligned}
$$

Table 4.4 : Result of classical and rapid carbohydrate assimilation test for C. parapsilosis $T o t a l=10$ strain (two standard strain included)


ND

| sucrose | $10,2 m a$ | 10 | 0.00 | ND |
| :--- | :--- | :--- | :--- | :--- |
| maltose | $24 \pi \Omega m$ | 10 | 0.00 | ND |


| llobios | 8 | 20.00 | . 56 |
| :---: | :---: | :---: | :---: |
| trehalose | 0 | 100.00 | 16.20 |NDND ND

$$
\begin{array}{lllll}
\text { raffinose } & 10 & 10 & 0.00
\end{array}
$$

ศษย่วิทยทรัพยาคร


$$
\begin{aligned}
& \text { DIFF }=\% \text { Difference } \\
& \text { ND }=\text { Not done }
\end{aligned}
$$

Table 4.5 : Result of classical and rapid carbohydrate assimilation test for Cr . neoformans Total $=52$ strain lone standard strain included)


$$
\begin{aligned}
& \% \text { DIFF }=\% \text { Difference } \\
& \text { ND } \quad=\text { Not done }
\end{aligned}
$$



Table 4.7 : Result of classical and rapid carbohydrate assimilation test for $\underline{R h}$. graminis Total $=9$ strain


[^0]lactose. For T. glabrdonshinu ${ }^{\text {Th }}$. trehalose but could not ferment galactose, sucrose, maltose and
 galactose and all of C. parapsiolsis could not ferment sucrose, maltose, trehalose and ladtose. For C. pseudotropicalis, all isolates could ferment galactose ancmaltose but could not ferment maltose, trehalose and laetose. Other isolates in the genus Candida had fit fermentation rofghton as in the standard key (Table 3.1).

Urease test

All of 327 isol'ated yeasts had been tested for comparative study of conventional angckapd yreage test. All of isolated yeasts in the genus Trichosporgpy, Rbodotoryla, Cryptococcus neoformans and the standard strains of these 3 yenera werepositive for urease by using conventionm and rapid urease testy There were no false positive and false negative in the rapid urease swab test
ศนยวิทยทรัพยากร

## Nitrate assimilation test

จุฬาลงกรรณ์มหาวิทยาลัย
A11 of 321 isolated yeasts had been tested for comparative study of conventional and rapid nitrate assimilation. The positive nitrate utilization yeast in the isolated yeast were Tr. pullulans and R. glutinis and also with standard strain of Cr . albidus. The result of rapid nitrate swab test and the conventional method were concomitant. In the rapid test, we could not see false positive or false negative result.

```
Growth in Sabouraud broth for film surface production
```

All isolates of Trichosporon had width film surface production while isolates of Rhodotorula and Cryptococcus had thin film surface production. One standard strath of Geotrichum had width film surface production. In the genus Candide) krusei (all strains gave the same result as Trichosporon. Fourty-nine ( $96.08 \%$ ) of 51 isolates of C. tropicalis had thin ifrm/surface production. For 18 of 142 C. albicans ( $12.68 \%$ ) produce $f 1 \mathrm{~m}$ surface. Other isolates in the genus Candida had the same result as-C. albicans.

Temperature tolerance


The three differentotomperatures $25^{\circ} \mathrm{C}, \quad 37^{\circ} \mathrm{C}$ and $42^{\circ} \mathrm{C}$ were selected to use in this investigation. All jeglates of C. albicans and T. glabratefould grow at $42^{\circ} \mathrm{C}$. For tropicalis, 49 of 51 isolates had growth at $42^{\circ} \mathrm{C}$. Other isolates in Candida had no growth 6 a
 isolates could not grow at $42^{\circ} \mathrm{C}$. but grow at $37^{\circ} \mathrm{C}$. One isolate of Tr .
 For Rhodotorula, All isolates could grow at $37^{\circ} \mathrm{C}$ but at $42^{\circ} \mathrm{C}$ it could not grow.

Germ tube test

On the study of germ tube production for rapid presumptive identification of C . albicans in pooled human serum, it was found that

Table 5. Result of other rapid methods compared with classical method
 isolated yeasts could not produce germ tube in this study.

## Chlamydoconidia production

Chlamydoconidia profudtion for rapid presumptive identification of C. albicans on ghatinous sice agar in 24 hours. There were 132 from 321 isolated yeasts posilive for ch amydoconidia and 131 (90.35\%) isolates were C. abigens and and species of the isolated, yasik were positive for chlamydoconidia in this study.


The resistance to cycloheximide could be used for rapid differentifeto of somequinich sight feant peasjs. on this study we had found that $142(97.93 \%)$ isolates of c. albicans were resistance to $0.1 \%$ aychexiotae and the z rewainder were serfsifig. Wone isolate of c. guilliermondii was resistant to $0.1 \%$ cycloheximide. All of other species and genera were sensitive to $0.1 \%$ cycloheximide.

Pigmentation from L-DOPA paper strip test

L-DOPA paper strip test for pigment formation was used in rapid identification of Cr. neoformans. All of isolated Cr.
neoformans and standard strain had produced dark pigment when streak these yeasts on the L-DOPA paper strip after 60 minutes. No other genera and species of isolated yeasts could produce dark pigment on the L-DOPA paper. For the two standard strains of Cr . albidus and Cr . laurentii also could not producelderk pigment on the L-DOPA paper.

All the Geyptococress standerd strains were positive for encapsulated yeast, $2 t$ was found thet only Cr. neoformans could produce capsule whion gould ${ }^{\text {agogelsily }}$ detected by this simple method. The other isolated yeast coutc not prcauce capsule especially for $C$. albicans, the most common 1 solatod yeast in this study.

After all of 321 isolated yeasts were inoculated in Sabouraud 6 a
 and fourty-five $(88.97 \%)$ of $C$. albicans had growth. One $c$.
 isolates of C. tropicalis had the same growth equal to $C$. albicans. The other isolated yeasts showed negative growth in Sabouraud broth pH 1.5.


[^0]:    \% DIFF $=$ \% Difference
    ND $\quad=$ Not done

