Chapter 4

DISCUSSION AND CONCLUSION

In this study it was found that the most common yeast in clinical laboratory is C. albicans (44.24%). It was found that uncommon species of of Candida, C. catenulata and C. lambica could be also isolated from clinical specimens (Table 2.) corresponding to the reports by Rippon and McGinnis (3,40). Yeasts in the genus Cryptococcus, only Cr. neoformans had been isolated and was rapidly differentiated by using L-DOPA paper strip test for pigmentation formation. In the genus Trichosporon, two isolated species were Tr. cutaneum (8.41%) and Tr. pullulans (0.31%) which also had reported in clinical specimens (3). The last genus Rhodotorula, three isolated species were R. rubra (1%), R. graminis (2.80%) and R. glutinis (0.31%), R. rubra may be a causative agent of systemic infection which was very rare case (7). In this study it was not found Geotrichum, the causative agent of geotrichosis.

In our germ tube production study, only C. albicans showed the growth of the tube in the incubation condition of 2 1/2 hours at 37°C, which is in the same range of other studies (89-100%) (40,52,56,58). Not only C. albicans produces germ tube, C. tropicalis could also demonstrate the tube with the low incidence (15%) (83). In this result, however, C. tropicalis showed the absent of the germ tube. Various media and incubation times were tested by some investigators. For example, Warwood and Blazevic (83) found that the production was

easier detected at 37°C than at 35°C on rice-cream agar. On the various media study, trypticase soy broth, 1% bacto peptone, sheep serum, plasma, egg white, saliva, cerebrospinal fluid, tissue culture medium TC 199 and pooled human serum were employed. Among these media, the pooled human serum was more practical to use since it gave the high yeild of the production and the serum was easily found in the medical laboratory. It is important, however, to recognize that germ tube production should not be the sole criterion for identifying C. albicans but that identification must be supported by other morpholigical and physiological tests (52).

Apart form the germ tube production, another important morphological study is chlamydoconidia production which is one of the criteria to differentiate C. albicans from other medically important yeasts. However, this kind of conidia is also found in C. tropicalis in the low rate (1-5%) (26,40,56). In the same as described above, chlamydoconidia was found in C. albicans (90.35%) and C. tropicalis (1.96%). The chlamydoconidia production was described in various medium such as rice infusion oxgal-tween 80 agar, zein-lactose-tween 80 agar, corn meal agar and glutineous rice tween 80 agar (40). Among these medium, glutineous rice tween 80 was suitable for routine laboratory because it was inexpensive and the chlamydoconidia could be easily observed.

In the rapid urease test and potassium nitrate assimilation test, it was found that the treated swab could be dried in normal air dry (see appendix). It was not necessary to lyophilized them as in the original paper(42,43). The obtainable results were satisfactory when ccmpared with the coventional methods. The swab could also be kept for 6 months in refrigerator with still produced the gcod result. These tests saved the time in preparation of medium ard increasing the space for other uses in laboratory. They are useful for differentiation yeast in genus Cryptococcus, Trichosporon, and Rhodotorula.

In the study of growth in acidic pH(pH 1.5) Sabouraud broth, it was found that 90.84% of C. albicans could grow and C. parapsilosis (0.31%) grew on this pH but no isolate of C. krusei grew on this pH as found in the later study by Odd, and Abbott, (28). So this test was useful in rapid differentiation of C. albicans.

By using the L-DOPA as a substrate for study the pigment formation, only Crytococcus neoformans can produced dark pigment. No flase positive result was detected. These results showed no different from others'(66,69,73,74). In addition for refreshment of L-DOPA paper before performing the test, distilled water was used instead of phosphate buffer (see appendix) and the yeild was the same like the previous study (89). From the above evidences, this modified test is recommended to differentiate Cr. neoformans from other genus and species in routine laboratory.

To study the cycloheximide resistance, the test was performed by using the drug concentration (0.1%) since the final concentration of cycloheximide in medium for using in routine isolation of yeast was 0.05% and the 0.1% drug concentration was recommended for the differentiate some species of Candida (59, 60). The result showed that most of C. albicans (90.84%), C. guilliermondii (100%, 1 strain) and some of C. pseudotropicalis (10%) resist the drug. And none of any isolate of C. tropicalis was positive for this test which was similar to the reports by Frienrich and Barnet, et al (59,60). This test, thus, could be used as a presumtive test to differentiate C. albicans from C. tropicalis and from the other Candida spp.

The complete carbohydrate assimilation reaction yielded by classical method took 72 hours, anyhow, some could detect positive results in 24 or 48 hours. So, the rapid method which took 18 hours for the complete results was very useful for rapid diagnosis and for the treatment. The results of rapid carbohydrate assimilation were concomitantly correctly with the conventional method for C. krusei, T. glabrata, and for C. tropicalis and Tr. cutaneum at p=0.01. In Candida albicans, cellobiose was assimilated in the rapid test, while it was not used in the conventional method. It had been suggested that cellobiose was susceptible to acid hydrolysis (9). The acid could degrade cellobiose to simple sugar which lead to false positive results. The acid contamination would come from the inoculum. This may the cause of false positive for arabinose assimilation too.

The reason why the false negative reaction of trehalose and xylose assimilation occured might be the insufficient incubation period and or the solidity of the medium. Syverson (86) also suggested that the factors affected on the results of assimilation were duration of incubation, temperature and the medium used in the test (broth or solid).

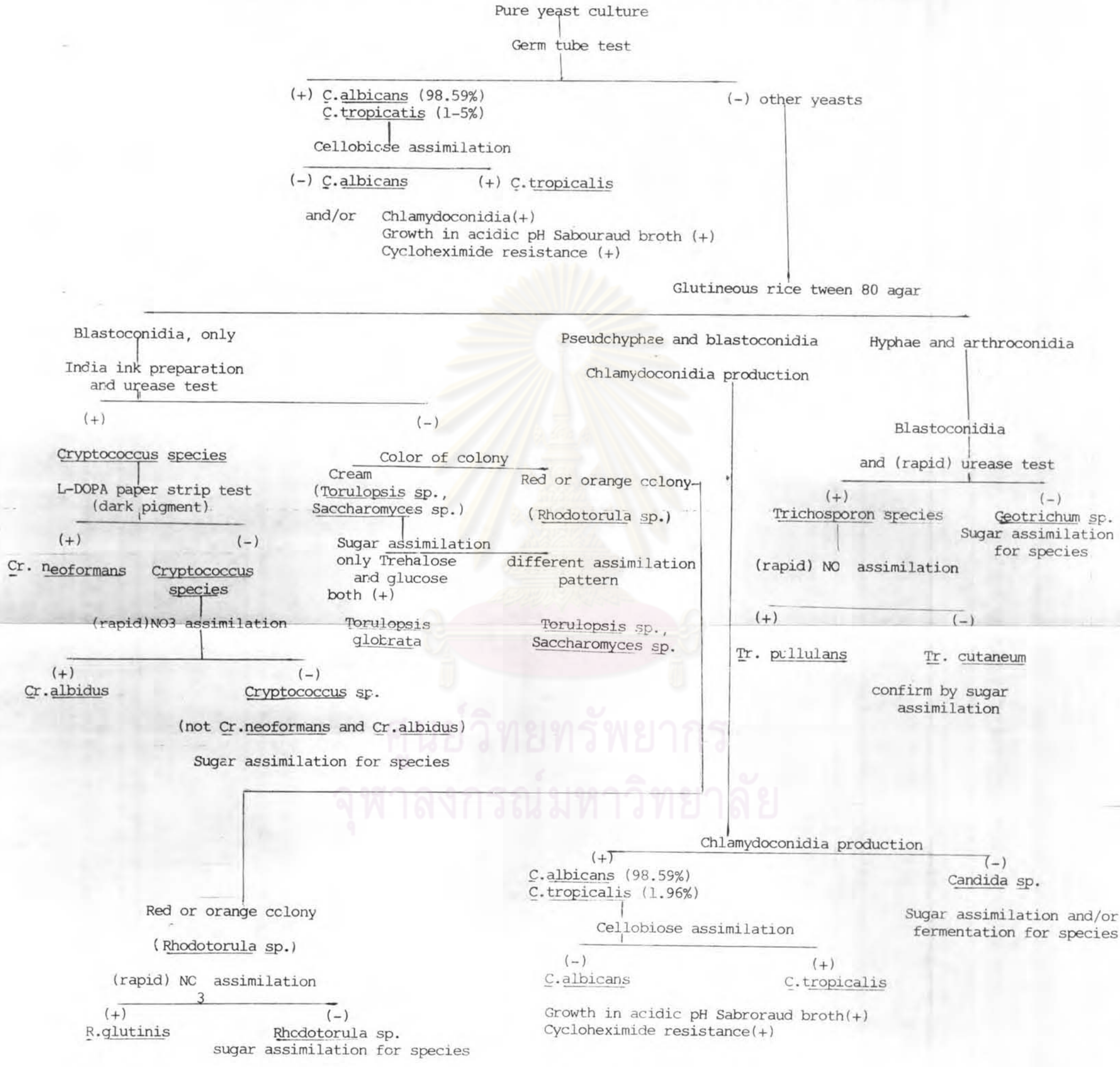
Apart from the above results, the recommended flow chart No. 1 was presented which was modified from Beneke (28) in some details. The first, after performing the germ tube production, assimilation is done for differentiation between C. albicans and C. tropicalis. Second, the use of rapid urease and nitrate assimilation were instead of classical method. Third, the caffeic acid reaction was replaced by modified L-DOPA paper strip test. Finally, the carbohydrate used on the assimilation test for differentiate the medically important yeasts between the genus Candida and Torulopsis glabrata were also recommended in the table 6.

In conclusion, all rapid tests, except the carbohydrate assimilation, have the same result as the classical method. But the former gave the higher reproducibility and consumed less than the latter. Moreover, it is easy to prepare, has extent shelf-life and low cost.

On the rapid carbohydrates assimilation test,it could be used in some species, but for routine laboratory usage, further development and more investigations are necessary. However, by using rapid

assimilation method it took only 18 hours for getting result, 3.2 ml. of yeast nitrogen base broth, and decrease 25 times the amount of each carbohydrate used per identification. So, for all preparations, micro-techique was or is cheaper than the conventional method. The further study would solve the problems of unconcomitant results of some carbohydrates which are assimilated i.e., cellobiose, trehalose, inositol, and dulcitol In addition, it should be concentrated on avoiding acid contamination from inoculum and providing modification of basal medium for good result and easy to handing for example by adding agar.

Chart 1 : The recommended chart for identification of medically important yeasts*



* Mcdified from Beneke (28)

Table 6. The recommended carbohydrate for differentiate yeast in the genus Candida and Troulopsis glabrata

Species	cellobiose	trehalose	lactose	melibiose	raffinose	soluble starch	dulcitol
C. albicans	-	+	-	-	-	+	-
C. tropicalis	+	+	-	-	-	+	-
C. pseudotropicalis	-	+	+	-	+	-	-
C. parapsilosis	-	+	-	-	-	-	-
C. guilliermondii	+	+	-	+	+	-	+
C. krusei	-	-	-	-	-	-	-
T. glabrata	-	+	-	-	-	-	-