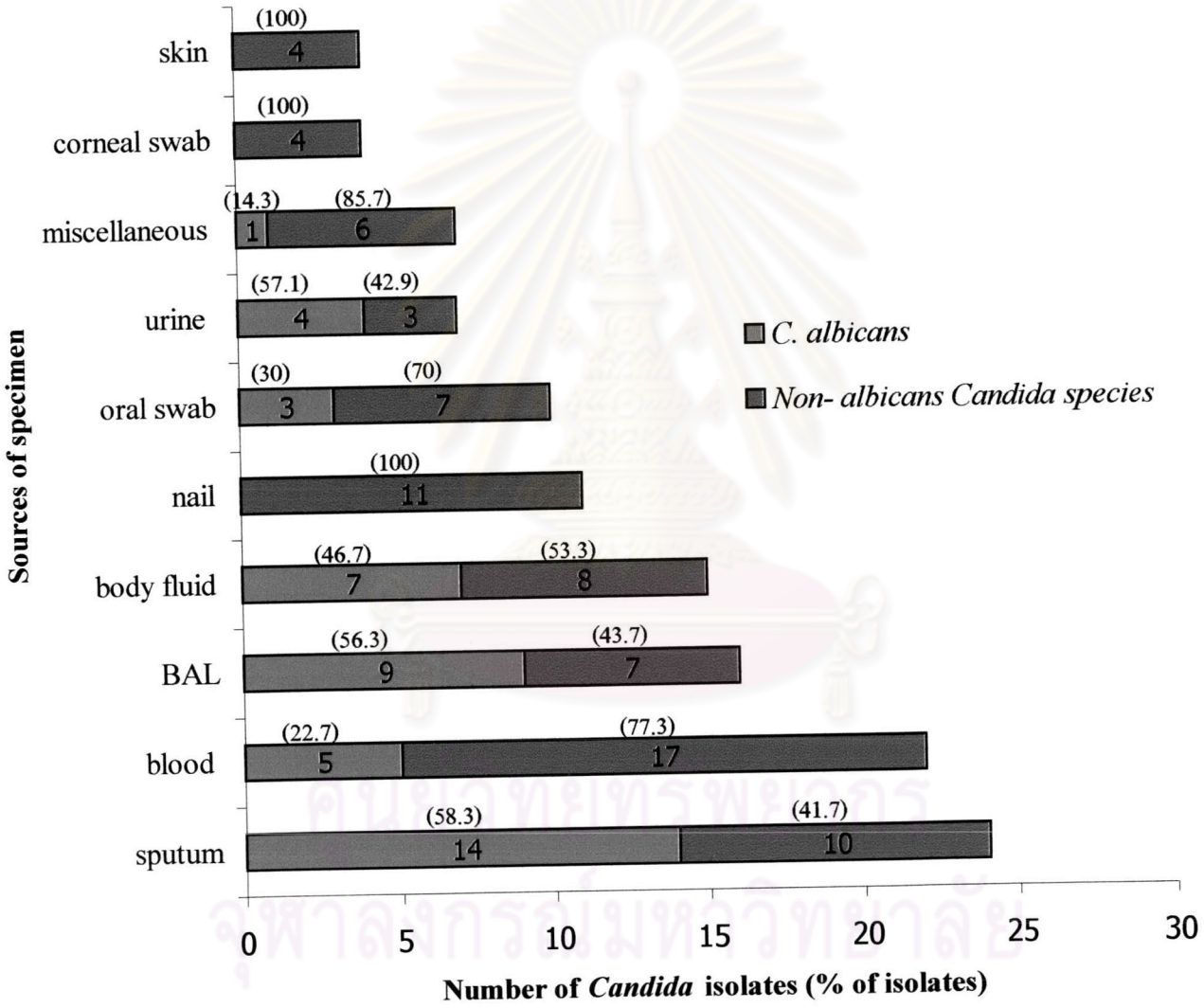
CHAPTER V

RESULTS

1. Clinical isolates

A total of 120 *Candida* isolates collected from stock cultures, Mycology Unit, Department of Microbiology, King Chulalongkorn Memorial Hospital, were used in this study. All *Candida* species were isolated from various specimens; 24 (20.0 %) isolates from sputum, 22 (18.3 %) blood, 16 (13.3 %) bronchial alveolar larvage (BAL), 15 (12.5 %) body fluid, 11 (9.2 %) nail, 10 (8.3 %) oral swab, 7 (5.8 %) urine, 4 (3.3 %) corneal swab, 4 (3.3 %) skin and 7 (5.8 %) miscellaneous (2 puses, 2 stools, 2 tissues, and 1 swab from foot). These cultured specimens were obtained from 74 male and 46 female patients. Of 120 isolates were identified as 43 (35.8%) *C. albicans* and 77 (64.2%) Non- *albicans* *Candida* species (NAC) by routine laboratory of Mycology Unit. The routine identification is based on the microscopic morphological examination, hyphae, chlamydoconidia production. Figure 9 showed the type of clinical specimens and the number of isolates that reported by routine identification during January to October 2002.

Figure 9. Sources and number of clinical specimens, which *Candida* was isolated during January to October 2002 from Mycology Unit, King Chulalongkorn Memorial Hospital



2. Identification of *Candida* species by conventional method

Identification the strains of *Candida* species have been previous performed in Mycology Unit of Microbiology Department, King Chulalongkorn Memorial Hospital (Fig.9). However, all strains were verified by conventional methods, i.e.; Reynold Brown phenomenon (germ tube production), chlamydoconidia production, and carbohydrate assimilations and carbohydrate fermentations (Table 14). These sixty-one isolates produced chlamydoconidia on glutinous rice agar within 48-72 hrs. All of these cultures except one demonstrated the ability to produce germ tube within 3 hrs. in serum at 35°C. Furthermore, the carbohydrate assimilation and carbohydrate fermentation was done. The results showed that eighty-two isolates was corresponded to those yeasts identification table (Beneke and Roger) (Fig. 10, Table. 15). The other 38 isolates were unable to identify because of the uncorresponding of the carbohydrate assimilation and fermentation (Table. 16). These isolates were further speciated by API 20C AUX (bioMerieux, France). Thus, based on the identification by conventional method plus commercial kit (API 20C AUX, bioMerieux, France) all the 120 isolates were listed in Table 14. In detail, there were 61 (50.8 %) *C. albicans,* 26 (21.7 %) *C. tropicalis*, 19 (15.8 %) *C. parapsilosis*, 9 (7.5 %) *C. glabrata*, 3 (2.5 %) *C. guilliermondii*, and 2 (1.7 %) *C. krusei* (Fig. 10). In clinical specimens, not only *C. albicans* was found but also the other species was isolated (Fig9). *C. albicans* was found mostly in oral swab (100%), sputum (79.1%), BAL (62.5%), body fluid (53.2%), skin (50%), miscellaneous (42.9%), blood (31.8%), urine (14.3%) and nail (9.1%) orderly. *C. tropicalis,* the second common species, was found mostly in urine (71.4%), miscellaneous (42.9%), blood (40.9%), body fluid (20%), BAL (12.5%), and sputum (8.2%), respectively. The third common species, *C. parapsilosis*, was isolated from most of the specimens especially in nail (81.8%), skin (50%), corneal swab (50%), miscellaneous (14.2%), blood (9.1), body fluid (6.7%), BAL (6.2%) and sputum (4.5%). *C. glabrata* was found in BAL (18.8%), urine (14.3%) , blood (9.1%), sputum (8.2%), body fluid (6.7%). *C. guilliermondii* was found only in blood and body fluid with 9.1% and 6.7 %, respectively. For 2 isolates of *C. krusei* was found in each nail (9.1%) and body fluid (6.7%) (Fig. 11). Table 17 showed distribution of *Candida* species, which results from routine and conventional method in clinical specimens and distribution of *Candida* species which results from *Candida* commercial kit (API 20C AUX, bioMerieuz, France) in clinical specimens were showed in Table 18.

Table 14. The results of 120 *Candida* isolates by conventional method and *Candida* commercial kit (API 20C AUX).

No.	Strain no.	Specimens	Germ tube	Chlamydoconidia	Carbohydrate assimilation												Carbohydrate Fermentation						Identification by Biochemical	Identified by API 20C AUX	% identity by API 20C AUX
					Glucose	Maltose	Sucrose	Lactose	Galactose	Melibiose	Cellobiose	Inositol	Xylose	Raffinose	Trehalose	Dulcitol	Glucose	Maltose	Sucrose	Lactose	Galactose	Trehalose			
1	IC-1	Nail	-	-	+	+	+	-	+	-	-	-	+	-	+	-	AG	-	-	-	-	Aw	*Unidentified*	*C.parapsilosis*	97%
2	IC-2	Nail	+	+	+	+	+	-	+	-	-	-	+	-	+	-	AG	AG	Aw	-	A	A	*C.albicans*		
3	IC-3	Body Fluid	-	-	+	-	-	-	-	-	-	-	-	-	+	-	AG	-	-	-	-	A	*C.glabrata*		
4	IC-4	Blood	-	+	+	+	+	-	+	-	-	-	+	-	+	-	-	-	A	-	A	-	*Unidentified*	*C.albicans*	83.20%
5	IC-5	BAL	-	-	+	-	-	-	-	-	-	-	-	-	+	-	AG	-	-	-	-	A	*C.glabrata*		
6	IC-6	Blood	+	+	+	+	+	-	+	-	-	-	+	-	+	-	AG	AG	A	-	Aw	Aw	*C.albicans*		
7	IC-7	BAL	-	-	+	+	+	-	+	-	+	-	+	-	+	-	AG	AG	AG	-	AG	AG	*C.tropicalis*		
8	IC-8	BAL	-	-	+	-	-	-	-	-	-	-	-	-	+	-	AG	-	-	-	-	A	*C.glabrata*		
9	IC-9	Nail	-	-	+	+	+	-	+	-	-	-	+	-	+	-	AG	-	-	-	-	-	*Unidentified*	*C.parapsilosis*	93.70%
10	IC-10	Blood	+	+	+	+	+	-	+	-	-	-	+	-	+	-	AG	AG	-	-	A	A	*Unidentified*	*C.albicans*	97.30%
11	IC-11	Urine	-	-	+	+	+	-	+	-	+	-	+	-	+	-	AG	AG	AG	-	AG	AG	*C.tropicalis*		
12	IC-12	Nail	-	-	+	+	+	-	+	-	-	-	+	-	+	-	AG	-	-	-	AG	A	*C.parapsilosis*		
13	IC-13	Sputum	+	+	+	+	+	-	+	-	-	-	+	-	+	-	AG	AG	A	-	A	A	*C.albicans*		
14	IC-14	Nail	-	-	+	-	-	-	-	-	-	-	-	-	-	-	AG	Aw	-	-	A	Aw	*Unidentified*	*C.krusei*	96.20%
15	IC-15	Sputum	-	-	+	-	-	-	-	-	-	-	±	-	+	-	AG	-	-	-	-	AG	*Unidentified*	*C.glabrata*	91.50%
16	IC-16	Urine	+	+	+	+	+	-	+	-	-	-	+	-	+	-	AG	AG	A	-	A	A	*C.albicans*		
17	IC-17	Body Fluid	-	-	+	+	+	-	+	-	+	-	+	-	+	-	AG	A	A	-	A	A	*Unidentified*	*C.tropicalis*	88.10%

(cont.) Table 14. The results of 120 *Candida* isolates by conventional method and *Candida* commercial kit (API 20C AUX).

No.	Strain no.	Specimens	Germ tube	Chlamydoconidia	Carbohydrate assimilation												Carbohydrate Fermentation						Identification by Biochemical	Identified by API 20C AUX	% identity by API 20C AUX
					Glucose	Maltose	Sucrose	Lactose	Galactose	Melibiose	Cellobiose	Inositol	Xylose	Raffinose	Trehalose	Dulcitol	Glucose	Maltose	Sucrose	Lactose	Galactose	Trehalose			
18	IC-18	oral swab	+	+	+	+	+	-	+	-	-	-	+	-	+	-	AG	AG	A	-	A	A	*C.albicans*		
19	IC-19	Blood	+	+	+	+	+	-	+	-	-	-	+	-	+	-	AG	AG	-	-	A	A	*Unidentified*	*C.albicans*	97.30%
20	IC-20	Sputum	+	+	+	+	+	-	+	-	-	-	+	-	+	-	AG	AG	A	-	A	A	*C.albicans*		
21	IC-21	Oral swab	+	+	+	+	+	-	+	-	-	-	+	-	+	-	AG	AG	A	-	A	A	*C.albicans*		
22	IC-22	Sputum	+	+	+	+	+	-	+	-	-	-	+	-	+	-	AG	AG	-	-	A	Aw	*Unidentified*	*C.albicans*	97.30%
23	IC-23	Sputum	+	+	+	+	+	-	+	-	-	-	+	-	+	-	AG	AG	-	-	A	Aw	*Unidentified*	*C.albicans*	97%
24	IC-24	Sputum	+	+	+	+	+	-	+	-	-	-	+	-	+	-	AG	AG	-	-	A	AG	*Unidentified*	*C.albicans*	85.50%
25	IC-25	Sputum	+	+	+	+	+	-	+	-	-	-	+	-	+	-	AG	AG	A	-	A	A	*C.albicans*		
26	IC-26	Body Fluid	+	+	+	+	+	-	+	-	-	-	+	-	+	-	AG	AG	A	-	A	A	*C.albicans*		
27	IC-27	Sputum	+	+	+	+	+	-	+	-	-	-	+	-	+	-	AG	AG	A	-	A	A	*C.albicans*		
28	IC-28	Sputum	+	+	+	+	+	-	±	-	-	-	+	-	+	-	AG	A	Aw	-	AW	Aw	*Unidentified*	*C.albicans*	95.50%
29	IC-29	Sputum	+	+	+	+	+	-	+	-	-	-	+	-	+	-	AG	AG	A	-	A	A	*C.albicans*		
30	IC-30	Corneal	-	-	+	+	+	-	+	-	+	-	+	-	+	-	AG	AG	AG	-	AG	AG	*C.tropicalis*		
31	IC-31	BAL	-	-	+	+	+	-	+	-	+	-	+	-	+	-	AG	AG	AG	-	AG	AG	*C.tropicalis*		
32	IC-32	Body Fluid	+	+	+	+	+	-	+	-	-	-	+	-	+	-	AG	AG	A	-	A	A	*C.albicans*		
33	IC-33	Body Fluid	+	+	+	+	+	-	+	-	-	-	+	-	+	-	AG	AG	-	-	A	A	*Unidentified*	*C.albicans*	97.30%
34	IC-34	BAL	+	+	+	+	+	-	+	-	-	-	+	-	+	-	-	AG	A	-	A	AG	*Unidentified*	*C.albicans*	95.30%

(cont.) Table 14. The results of 120 *Candida* isolates by conventional method and *Candida* commercial kit (API 20C AUX).

No.	Strain no.	Specimens	Germ tube	Chlamydoconidia	Carbohydrate assimilation												Carbohydrate Fermentation						Identification by Biochemical	Identified by API 20C AUX	% identity by API 20C AUX
					Glucose	Maltose	Sucrose	Lactose	Galactose	Melibiose	Cellobiose	Inositol	Xylose	Raffinose	Trehalose	Dulcitol	Glucose	Maltose	Sucrose	Lactose	Galactose	Trehalose			
35	IC-35	BAL	+	+	+	+	+	-	+	-	-	-	+	-	+	-	AG	AG	A	-	A	A	*C.albicans*		
36	IC-36	Body Fluid	+	+	+	+	+	-	+	-	-	-	+	-	+	-	AG	AG	-	-	A	A	*Unidentified*	*C.albicans*	97%
37	IC-37	BAL	+	+	+	+	+	-	+	-	-	-	+	-	+	-	AG	AG	-	-	-	-	*Unidentified*	*C.albicans*	91.30%
38	IC-38	Blood	+	+	+	+	+	-	+	-	-	-	+	-	±	-	AG	A	-	-	Aw	A	*Unidentified*	*C.albicans*	92.30%
39	IC-39	Nail	-	-	+	+	+	-	+	-	-	-	+	-	+	-	AG	-	-	-	AG	A	*C.parapsilosis*		
40	IC-40	Sputum	+	+	+	+	+	-	+	-	-	-	+	-	+	-	AG	AG	A	-	A	A	*C.albicans*		
41	IC-41	Body Fluid	-	-	+	-	-	-	-	-	-	-	-	-	-	-	AG	Aw	-	-	Aw	Aw	*Unidentified*	*C.krusei*	97.30%
42	IC-42	Sputum	+	+	+	+	+	-	+	-	-	-	+	-	+	-	AG	AG	A	-	A	A	*C.albicans*		
43	IC-43	Sputum	-	-	+	-	-	-	-	-	-	-	-	-	+	-	AG	-	-	-	-	A	*C.glabrata*		
44	IC-44	Pus	-	-	+	+	+	-	+	-	+	-	+	-	+	-	AG	A	AG	-	Aw	AG	*Unidentified*	*C.tropicalis*	95.80%
45	IC-45	BAL	+	+	+	+	+	-	+	-	-	-	+	-	+	-	AG	AG	-	-	Aw	Aw	*Unidentified*	*C.albicans*	97%
46	IC-46	Oral swab	+	+	+	+	+	-	+	-	-	-	+	-	+	-	AG	AG	A	-	A	A	*C.albicans*		
47	IC-47	Body Fluid	+	+	+	+	+	-	+	-	-	-	+	-	+	-	AG	AG	A	-	A	A	*C.albicans*		
48	IC-48	Sputum	+	+	+	+	+	-	+	-	-	-	+	-	+	-	AG	AG	A	-	A	A	*C.albicans*		
49	IC-49	Blood	-	-	+	+	+	-	+	-	+	-	+	-	+	-	AG	AG	AG	-	AG	AG	*C.tropicalis*		
50	IC-50	Blood	+	+	+	+	+	-	+	-	-	-	+	-	+	-	AG	A	-	-	-	Aw	*Unidentified*	*C.albicans*	93.50%
51	IC-51	Blood	-	-	+	+	+	-	+	-	-	-	+	-	+	-	AG	-	-	-	AG	AG	*C.parapsilosis*		

(cont.) Table 14. The results of 120 *Candida* isolates by conventional method and *Candida* commercial kit (API 20C AUX).

No.	Strain no.	Specimens	Germ tube	Chlamydoconidia	Carbohydrate assimilation												Carbohydrate Fermentation						Identification by Biochemical	Identified by API 20C AUX	% identity by API 20C AUX
					Glucose	Maltose	Sucrose	Lactose	Galactose	Melibiose	Cellobiose	Inositol	Xylose	Raffinose	Trehalose	Dulcitol	Glucose	Maltose	Sucrose	Lactose	Galactose	Trehalose			
52	IC-52	Blood	+	+	+	+	+	-	+	-	-	-	+	-	+	-	AG	AG	A	-	A	A	*C.albicans*		
53	IC-53	Blood	-	-	+	+	+	-	+	-	+	-	+	+	+	-	AG	AG	AG	-	A	AG	*Unidentified*	*C.guilliermondii*	92.90%
54	IC-54	Sputum	+	+	+	+	+	-	+	-	-	-	+	-	+	-	AG	AG	A	-	A	A	*C.albicans*		
55	IC-55	BAL	+	+	+	+	+	-	+	-	-	-	+	-	+	-	AG	A	-	-	A	A	*Unidentified*	*C.albicans*	97.30%
56	IC-56	Sputum	-	-	+	+	+	-	+	-	+	-	+	-	+	-	AG	AG	AG	-	AG	AG	*C.tropicalis*		
57	IC-57	BAL	+	+	+	+	+	-	+	-	-	-	+	-	+	-	AG	AG	A	-	A	A	*C.albicans*		
58	IC-58	BAL	+	+	+	+	+	-	+	-	-	-	+	-	+	-	AG	AG	A	-	A	A	*C.albicans*		
59	IC-59	BAL	+	+	+	+	+	-	+	-	-	-	+	-	+	-	AG	AG	A	-	A	A	*C albicans*		
60	IC-60	Sputum	+	+	+	+	+	-	+	-	-	-	+	-	+	-	AG	AG	-	-	Aw	A	*Unidentified*	*C.albicans*	97.30%
61	IC-61	Nail	-	-	+	+	+	-	+	-	-	-	+	-	±	-	AG	-	-	-	-	A	*Unidentified*	*C.parapsilosis*	92.30%
62	IC-62	Body Fluid	-	-	+	+	+	-	+	-	-	-	+	-	+	-	AG	-	-	-	AG	A	*C.parapsilosis*		
63	IC-63	Body Fluid	+	+	+	+	+	-	+	-	-	-	+	-	+	-	AG	AG	A	-	A	A	*C.albicans*		
64	IC-64	Urine	-	-	+	+	+	-	+	-	+	-	+	-	+	-	AG	A	A	-	Aw	Aw	*Unidentified*	*C.tropicalis*	89.30%
65	IC-65	Tissue	-	-	+	+	+	-	+	-	+	-	+	-	+	-	AG	AG	AG	-	AG	AG	*C.tropicalis*		
66	IC-66	Swab from foot	-	-	+	+	+	-	+	-	+	±	+	-	+	-	AG	A	AG	-	A	A	*Unidentified*	*C.tropicalis*	97.30%
67	IC-67	Body Fluid	+	+	+	+	+	-	+	-	-	-	+	-	+	-	AG	AG	A	-	A	A	*C.albicans*		
68	IC-68	Body Fluid	+	+	+	+	+	-	+	-	-	-	+	-	+	-	AG	AG	A	-	A	A	*C.albicans*		

(cont.) Table 14. The results of 120 *Candida* isolates by conventional method and *Candida* commercial kit (API 20C AUX).

No.	Strain no.	Specimens	Germ tube	Chlamydoconidia	Carbohydrate assimilation												Carbohydrate Fermentation						Identification by Biochemical	Identified by API 20C AUX	% identity by API 20C AUX
					Glucose	Maltose	Sucrose	Lactose	Galactose	Melibiose	Cellobiose	Inositol	Xylose	Raffinose	Trehalose	Dulcitol	Glucose	Maltose	Sucrose	Lactose	Galactose	Trehalose			
69	IC-69	corneal	-	-	+	+	+	-	+	-	+	-	+	-	+	-	AG	AG	AG	-	AG	AG	*C.tropicalis*		
70	IC-70	Sputum	+	+	+	+	+	-	+	-	-	-	+	-	+	-	AG	A	-	-	Aw	A	*Unidentified*	*C.albicans*	96.50%
71	IC-71	Blood	-	-	+	+	+	-	+	-	+	-	+	-	+	-	AG	AG	AG	-	AG	AG	*C.tropicalis*		
72	IC-72	Pus	+	+	+	+	+	-	+	-	-	-	+	-	+	-	AG	AG	A	-	A	A	*C.albicans*		
73	IC-73	Urine	+	+	+	+	+	-	+	-	+	-	+	-	+	-	AG	AG	AG	-	AG	AG	*C.tropicalis*		
74	IC-74	Blood	-	-	+	+	+	-	+	-	+	-	+	-	+	-	AG	AG	AG	-	AG	AG	*C.tropicalis*		
75	IC-75	Blood	-	-	+	+	+	-	+	-	+	-	+	-	+	-	AG	AG	AG	-	AG	AG	*C.tropicalis*		
76	IC-76	Blood	-	-	+	-	-	-	-	-	-	-	-	-	+	-	AG	-	-	-	-	A	*C.glabrata*		
77	IC-77	Corneal	-	-	+	+	+	-	+	-	-	-	+	-	±	-	A	-	-	-	-	Aw	*Unidentified*	*C.parapsilosis*	93.30%
78	IC-78	Blood	-	-	+	+	+	-	+	-	+	-	+	-	+	-	AG	AG	AG	-	AG	AG	*C.tropicalis*		
79	IC-79	Urine	+	+	+	+	+	-	+	-	+	-	+	-	+	-	AG	AG	AG	-	AG	AG	*C.tropicalis*		
80	IC-80	oral swab	+	+	+	+	+	-	+	-	-	-	+	-	+	-	AG	AG	A	-	A	A	*C.albicans*		
81	IC-81	BAL	+	+	+	+	+	-	+	-	-	-	+	-	+	-	AG	A	Aw	-	A	Aw	*Unidentified*	*C.albicans*	97%
82	IC-82	Oral swab	+	+	+	+	+	-	+	-	-	-	+	-	+	-	AG	AG	A	-	A	A	*C.albicans*		
83	IC-83	Sputum	+	+	+	+	+	-	+	-	-	-	+	-	+	-	AG	AG	A	-	A	A	*C.albicans*		
84	IC-84	Stool	-	-	+	+	+	-	+	-	-	-	+	-	+	-	AG	-	-	-	AG	A	*C.parapsilosis*		
85	IC-85	BAL	+	+	+	+	+	-	+	-	-	-	+	-	+	-	AG	AG	A	-	A	A	*C.albicans*		

(cont.) Table 14. The results of 120 *Candida* isolates by conventional method and *Candida* commercial kit (API 20C AUX).

No.	Strain no.	Specimens	Germ tube	Chlamydoconidia	Carbohydrate assimilation												Carbohydrate Fermentation						Identification by Biochemical	Identified by API 20C AUX	% identity by API 20C AUX
					Glucose	Maltose	Sucrose	Lactose	Galactose	Melibiose	Cellobiose	Inositol	Xylose	Raffinose	Trehalose	Dulcitol	Glucose	Maltose	Sucrose	Lactose	Galactose	Trehalose			
86	IC-86	Blood	-	-	+	-	-	-	-	-	-	-	-	-	+	-	A	Aw	-	-	-	Aw	*Unidentified*	*C.glabrata*	96.50%
87	IC-87	Corneal	-	-	+	+	+	-	+	-	-	-	+	-	+	-	A	Aw	-	-	Aw	-	*Unidentified*	*C.parapsilosis*	95.30%
88	IC-88	Body Fluid	-	-	+	+	+	-	+	-	+	-	+	-	+	-	AG	AG	AG	-	AG	AG	*C.tropicalis*		
89	IC-89	BAL	-	-	+	+	+	-	+	-	-	-	+	-	+	-	AG	-	-	-	AG	Aw	*C.parapsilosis*		
90	IC-90	Sputum	-	-	+	+	+	-	+	-	+	-	+	-	+	-	AG	AG	AG	-	AG	AG	*C.tropicalis*		
91	IC-91	Oral swab	+	+	+	+	+	-	+	-	-	-	+	-	+	-	AG	AG	A	-	A	A	*C.albicans*		
92	IC-92	Nail	-	-	+	+	+	-	+	-	-	-	+	-	+	-	AG	-	-	-	-	Aw	*Unidentified*	*C.parapsilosis*	97%
93	IC-93	Stool	+	+	+	+	+	-	+	-	-	-	+	-	+	-	AG	AG	A	-	A	A	*C.albicans*		
94	IC-94	Oral swab	+	+	+	+	+	-	+	-	-	-	+	-	+	-	AG	AG	A	-	A	A	*C.albicans*		
95	IC-95	Oral swab	+	+	+	+	+	-	+	-	-	-	+	-	+	-	AG	AG	A	-	A	A	*C.albicans*		
96	IC-96	Body Fluid	-	-	+	+	+	-	+	-	+	-	+	-	+	-	AG	AG	AG	-	AG	AG	*C.tropicalis*		
97	IC-97	Oral swab	+	+	+	+	+	-	+	-	-	-	+	-	+	-	A	A	-	-	Aw	Aw	*Unidentified*	*C.albicans*	92.30%
98	IC-98	Body Fluid	-	-	+	+	+	-	+	+	+	-	+	+	+	+	AG	-	AG	-	A	A	*C.guilliermondii*		
99	IC-99	Nail	-	-	+	+	+	-	+	-	-	-	+	-	+	-	AG	-	-	-	AG	A	*C.parapsilosis*		
100	IC-100	Sputum	+	+	+	+	+	-	+	-	-	-	+	-	+	-	AG	AG	A	-	A	A	*C.albicans*		
101	IC-101	oral swab	+	+	+	+	+	-	+	-	-	-	+	-	+	-	AG	AG	A	-	A	A	*C.albicans*		
102	IC-102	Sputum	+	+	+	+	+	-	+	-	-	-	+	-	+	-	AG	AG	A	-	A	A	*C.albicans*		

(cont.) Table 14. The results of 120 *Candida* isolates by conventional method and *Candida* commercial kit (API 20C AUX).

No.	Strain no.	Specimens	Germ tube	Chlamydoconidia	Carbohydrate assimilation												Carbohydrate Fermentation						Identification by Biochemical	Identified by API 20C AUX	% identity by API 20C AUX
					Glucose	Maltose	Sucrose	Lactose	Galactose	Melibiose	Cellobiose	Inositol	Xylose	Raffinose	Trehalose	Dulcitol	Glucose	Maltose	Sucrose	Lactose	Galactose	Trehalose			
103	IC-103	Nail	-	-	+	+	+	-	+	-	-	-	+	-	+	-	AG	-	-	-	AG	A	*C.parapsilosis*		
104	IC-104	Nail	-	-	+	+	+	-	+	-	-	-	+	-	+	-	AG	-	-	-	-	Aw	*Unidentified*	*C.parapsilosis*	95.50%
105	IC-105	Sputum	+	+	+	+	+	-	+	-	-	-	+	-	+	-	AG	A	-	-	Aw	A	*Unidentified*	*C.albicans*	96.50%
106	IC-106	Blood	-	-	+	+	+	-	+	-	+	-	+	-	+	-	AG	AG	AG	-	AG	AG	*C.tropicalis*		
107	IC-107	Blood	-	-	+	+	+	-	+	-	+	-	+	-	+	-	AG	AG	AG	-	AG	AG	*C.tropicalis*		
108	IC-108	Blood	-	-	+	+	+	-	+	-	+	-	+	-	+	-	AG	AG	AG	-	AG	AG	*C.tropicalis*		
109	IC-109	Tissue	+	+	+	+	+	-	+	-	-	-	+	-	+	-	AG	AG	A	-	A	A	*C.albicans*		
110	IC-110	Blood	-	-	+	+	+	-	±	-	-	-	+	-	+	-	A	-	-	-	A	-	*Unidentified*	*C.parapsilosis*	96.80%
111	IC-111	Sputum	-	-	+	+	+	-	+	-	-	-	+	-	+	-	AG	-	-	-	AG	Aw	*C.parapsilosis*		
112	IC-112	Skin	-	-	+	+	+	-	+	-	-	-	+	-	±	-	AG	-	-	-	-	A	*Unidentified*	*C.parapsilosis*	93.30%
113	IC-113	Skin	-	-	+	+	+	-	+	-	-	-	+	-	+	-	AG	-	-	-	AG	A	*C.parapsilosis*		
114	IC-114	Skin	+	+	+	+	+	-	+	-	-	-	+	-	+	-	AG	AG	A	-	A	A	*C.albicans*		
115	IC-115	Skin	+	+	+	+	+	-	+	-	-	-	+	-	+	-	AG	AG	A	-	A	A	*C.albicans*		
116	IC-116	Blood	-	-	+	+	+	-	+	-	+	-	+	-	+	-	AG	AG	AG	-	AG	AG	*C.tropicalis*		
117	IC-117	Urine	-	-	+	-	-	-	-	-	-	-	-	-	+	-	AG	-	-	-	-	A	*C.glabrata*		
118	IC-118	Urine	-	-	+	+	+	-	+	-	+	-	+	-	+	-	AG	AG	AG	-	AG	AG	*C.tropicalis*		
119	IC-119	BAL	-	-	+	-	-	-	-	-	-	-	-	-	+	-	AG	-	-	-	-	A	*C.glabrata*		
120	IC-120	Blood	-	-	+	+	+	-	+	+	+	-	+	+	+	+	AG	-	AG	-	A	AG	*C.guilliermondii*		

Figure 10. *Candida* species identification by using conventional assay and *Candida* commercial kit (API 20C AUX, bioMerieux, France)

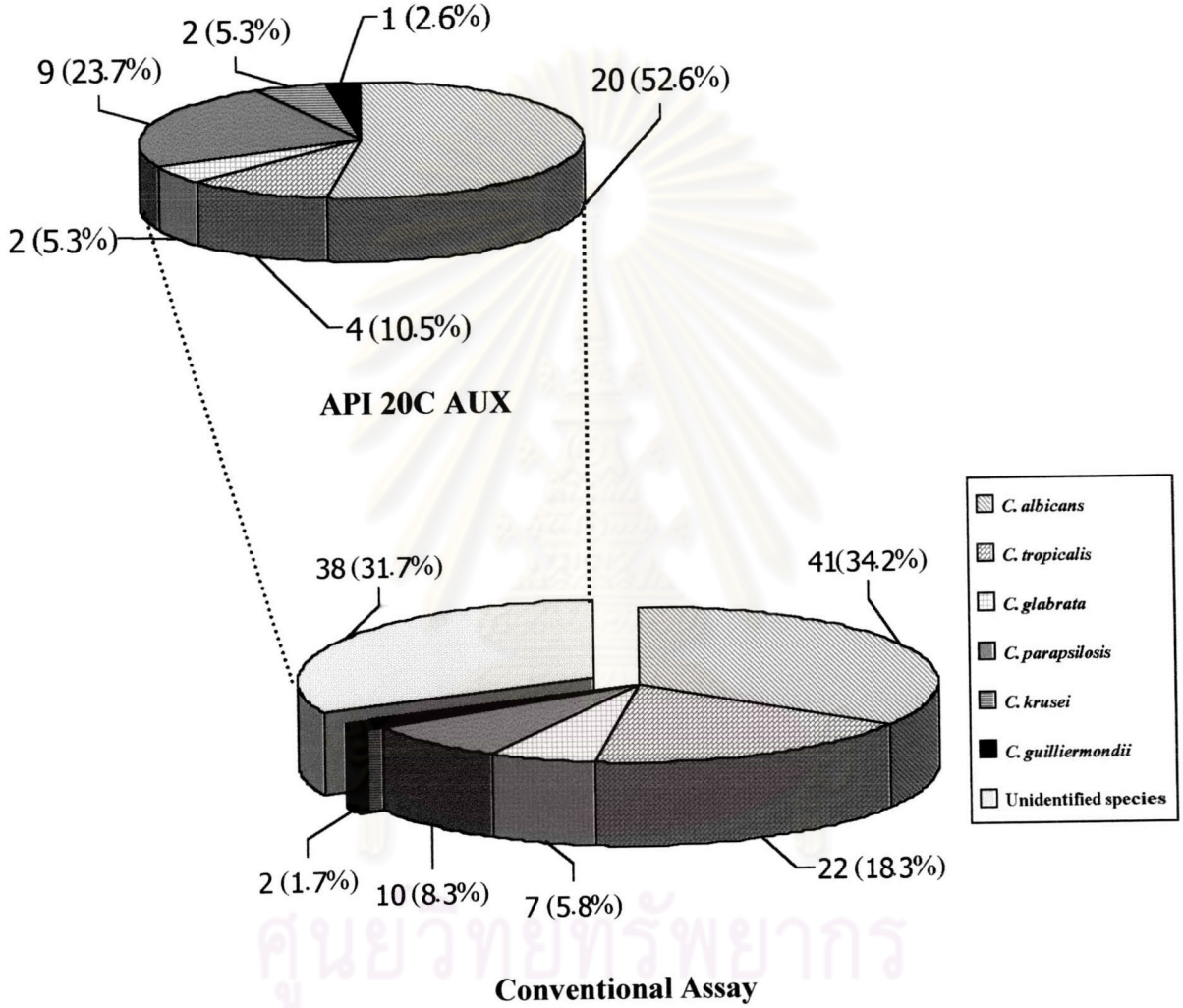


Figure 11. Sources and number of clinical specimens, which *Candida* was identified by conventional and *Candida* commercial kit (API 20C AUX, bioMerieuz, France)

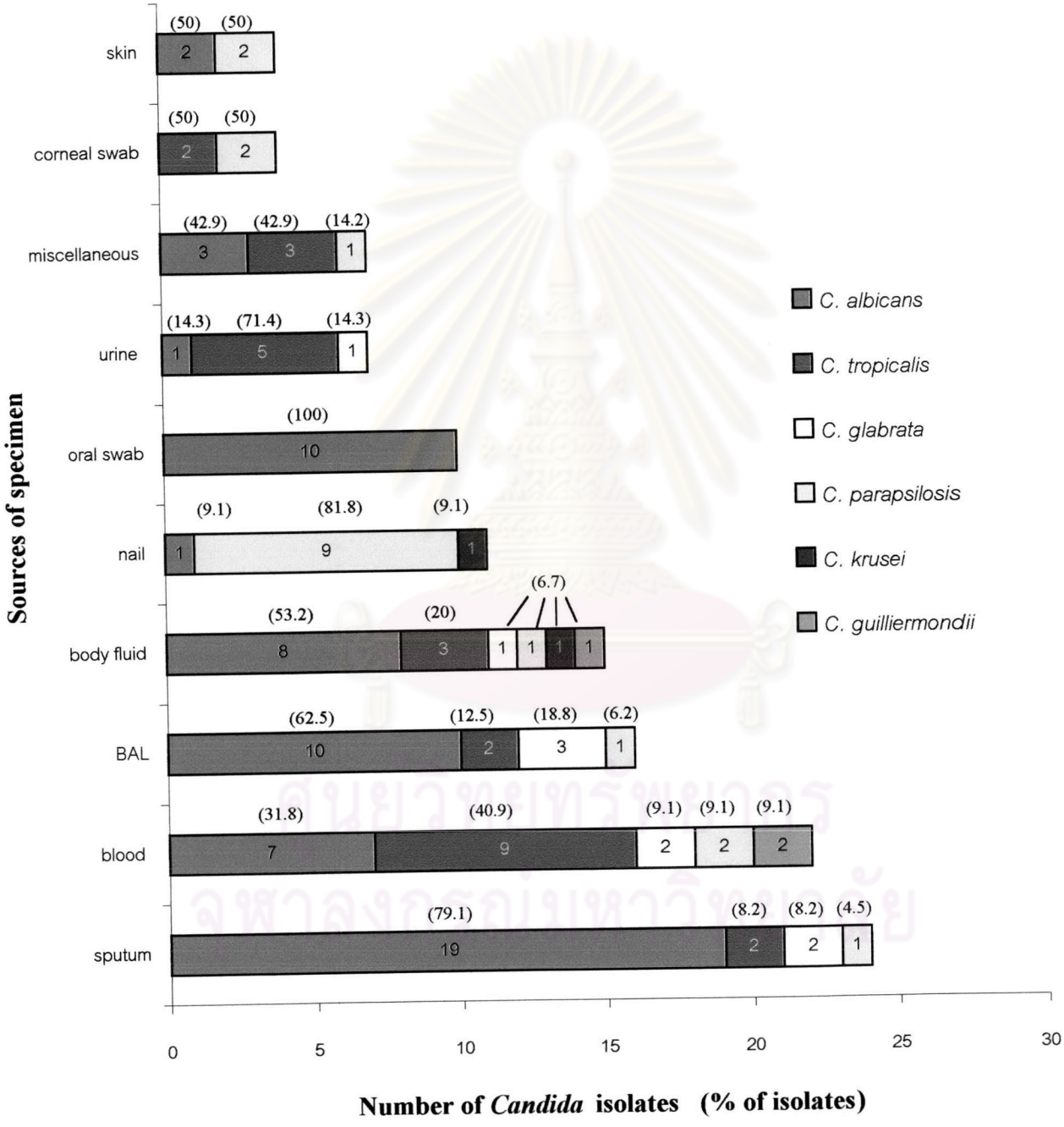


Table 15. All 120 *Candida* isolates were identified by conventional assay.

Species	No of isolates (%)
C. albicans	41 (34.2%)
C. tropicalis	22 (18.3%)
C. parapsilosis	10 (8.3%)
C. glabrata	7 (5.8%)
C. guilliermondii	2 (1.7%)
Unidentified species	38 (31.7%)
Total	120 (100%)

Table 16. 38 unidentified *Candida* isolates were identified by *Candida* commercial kit (API 20C AUX, bioMerieuz, France).

Species	No of isolates (%)
C. albicans	20 (52.6%)
C. tropicalis	4 (10.5%)
C. parapsilosis	9 (23.7%)
C. glabrata	2 (5.3%)
C. krusei	2 (5.3%)
C. guilliermondii	1 (2.6%)
Total	38 (100%)

Table 17. Distribution of *Candida* species which results from routine and conventional method in clinical specimens

Specimens	Routine Results			Conventional Results					
	Total	*C. albicans*	NAC	*C. albicans*	*C. glabrata*	*C. guilliermondii*	*C. parapsilosis*	*C. tropicalis*	Unidentified species
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
skin	4(3.3%)	0 (0%)	4(100%)	2(50%)	0 (0%)	0 (0%)	1 (25%)	0 (0%)	1 (25%)
corneal swab	4(3.3%)	0 (0%)	4(100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (50%)	2 (50%)
miscellenous	7 (5.8%)	1(14.3%)	6(58.7%)	3 (42.9%)	0 (0%)	0 (0%)	1 (14.3%)	1 (14.3%)	2 (28.6%)
urine	7(5.8%)	4(57.1%)	3(42.9%)	1(14.3%)	1 (14.3%)	0 (0%)	0 (0%)	4 57.1%)	1 (14.3%)
oral swab	10(8.3%)	3 (30%)	7 (70%)	9 (90%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (10%)
nail	11(9.2%)	0 (0%)	11 (100%)	1 (9.1%)	0 (0%)	0 (0%)	4 (36.4%)	0 (0%)	6 (54.5%)
body Fluid	15 (12.5%)	7(46.7%)	8 (53.3%)	6 (40%)	1 (6.7%)	1 (6.7%)	1 (6.7%)	2 (13.3%)	4 (26.7%)
BAL	16 (13.3%)	9 (56.3%)	7 (43.7%)	5 (31.3%)	3 (18.8%)	0 (0%)	1(6.3%)	2 (12.5%)	5 (31.3%)
blood	22 (18.3%)	5 (22.7%)	17 (77.3%)	2 (9.1%)	1 (4.5%)	1 (4.5%)	1 (4.5%)	9 (40.9%)	8 (36.4%)
sputum	24 (20%)	14 (58.3%)	10 (41.7%)	12 (50%)	1 (4.2%)	0 (0%)	1 (4.2%)	2 (8.3%)	8 (33.3%)
Total	120 (100%)	43 (35.8%)	77 (64.2%)	41(34.2%)	7 (5.8%)	2 (1.7%)	10 (8.3%)	22 (18.3%)	38 (31.7%)

NAC = Non- *albicans Candida* species

Table 18. Distribution of *Candida* species, which results from *Candida* commercial kit (API 20C AUX) in clinical specimens

Specimens	*C. albicans*	*C. tropicalis*	*C. glabrata*	*C. parapsilosis*	*C. krusei*	*C. guilliermondii*	Total
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
skin	0 (0%)	0 (0%)	0 (0%)	1 (100%)	0 (0%)	0 (0%)	1 (2.6%)
corneal swab	0 (0%)	0 (0%)	0 (0%)	2 (100%)	0 (0%)	0 (0%)	2 (5.3%)
urine	0 (0%)	1 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (2.6%)
miscellaneous	0 (0%)	2 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (5.3%)
oral swab	1 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (2.6%)
nail	0 (0%)	0 (0%)	0 (0%)	5 (83.3%)	1 (16.7%)	0 (0%)	6 (15.8%)
body fluid	2 (50%)	1 (25%)	0 (0%)	0 (0%)	1 (25%)	0 (0%)	4 (10.5%)
BAL	5 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	5 (13.2%)
blood	5 (62.5%)	0 (0%)	1 (12.5%)	1 (12.5%)	0 (0%)	1 (12.5%)	8 (21.1%)
sputum	7 (87.5%)	0 (0%)	1 (12.5%)	0 (0%)	0 (0%)	0 (0%)	8 (21.1%)
Total	20 (52.6%)	4 (10.5%)	2 (5.3%)	9 (23.7%)	2 (5.3%)	1 (2.6%)	38 (100%)

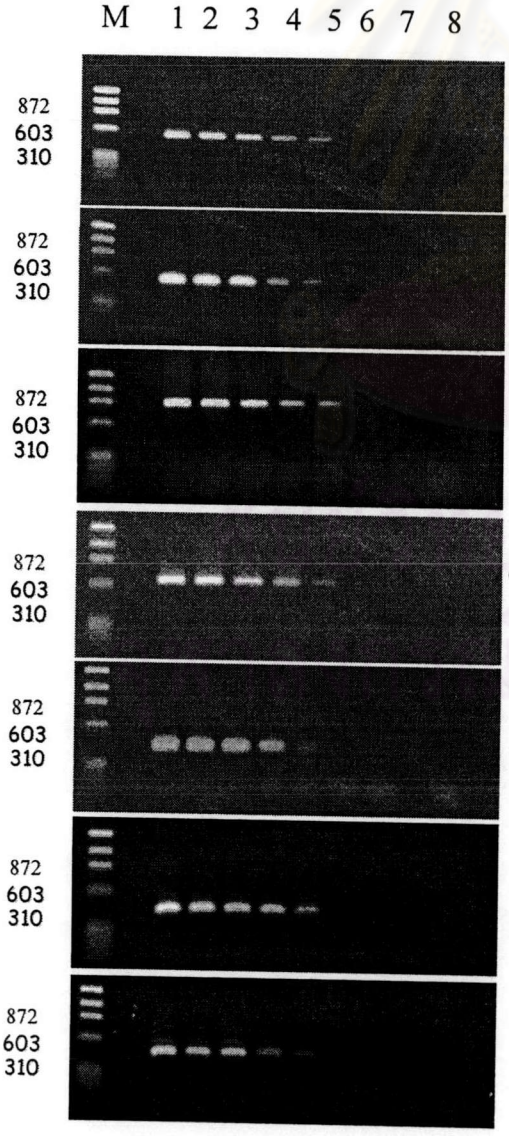
3. PCR amplification for detection of *Candida* species

3.1 Reference strains

3.1.1 Sensitivity for DNA detection

To analyze the sensitivity of PCR assay by using the ITS1 and ITS4 primers, all the diluted templates were performed the same conditions. The result showed that merely 10 *Candida* cells or 400 fg DNA of *C. albicans* was able to be detected. Not only four hundred femtograms at least amount of DNA of *C. albicans* but also the same amount of DNA of other six reference *Candida* strains were also detected. (Fig. 12)

Figure 12. Sensitivity of DNA detection by PCR of seven difference *Candida* DNA with ethidium bromide staining on agarose gel electrophoresis.



3.1.2 PCR and RFLP patterns of *Candida* species reference strains by using ITS 1 and ITS4 primers.

The intergenic spacer region was successfully amplified from all reference strains, and a distinct product size was consistently obtained for all reference strains of a given species. *Candida glabrata* yielded a unique product size of approximately 870 bp. Similarly, *C. guilliermondii* yielded product sizes of 600 bp (Fig. 13, Table 19). *C. guilliermondii and C. glabrata* showed species-specific differences in the sizes of the PCR amplified products. A product of approximately 520 bp was obtained with the remaining strains (Fig. 13). By the PCR merely was hardly differentiating the species. These PCR products were studied further by RFLP analysis following digestion of the PCR product by the restriction enzymes *Hae* III, *Dde* I, and *Tru9* I. Figure 13, 14, 15 and 16 showed a typical gel electrophoresis of PCR products obtained from seven *Candida* species and digested with *Hae* III, *Dde* I, and *Tru9* I restriction enzyme, respectively. Table 19, 20, 21, and 22 showed the PCR products and DNA fragments size that restricted by *Hae* III, *Dde* I, and *Tru9* I, of seven *Candida* species from GenBank, respectively that analyzed by Bioedit program. In the patterns of PCR products size, only *C. guilliermondii and C. glabrata* were speciated if it was products size as 600 bp and 800 bp, orderly whereas other five species were not identified by used only PCR product size.

The RFLP patterns of *Hae* III restriction enzyme analysis show 5 types of restriction fragments patterns. If product size that cut by this enzyme showed bands approximately 650 bp and approximately 220 bp was analyzed as *C. glabrata. C. guilliermondii* showed typical 3 bands which product size approximately 400 bp,118 bp, and 80 bp. *C. parapsilosis* showed 2 bands as approximately 400 bp, and 100 bp. This enzyme cut the DNA of *C. krusei* as three bands at approximately 380 bp, 90 bp, and 40 bp. For DNA of *C. albicans, C. tropicalis, and C. dubliniensis* showed 2 bands at approximately 450 bp and 90 bp that were cut with *Hae* III. The RFLP patterns of *Hae* III cannot speciate *C. albicans, C. tropicalis, and C. dubliniensis* because it gave the similar 2 bands of RFLP patterns at approximately 450 bp and 90 bp.

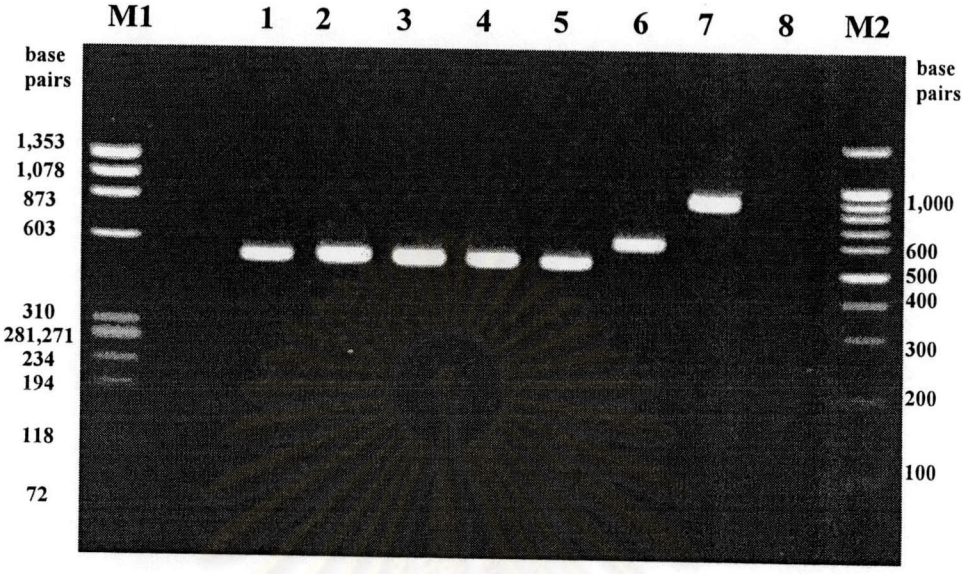
In *Dde* I pattern analysis, it showed five type patterns of RFLP; the first was in *C. glabrata* showed the band approximately 780 bp and 50 bp, the second pattern was in *C. guilliermondii* with band at approximately 380 bp and 210 bp, the third pattern was in *C. dubliniensis* that showed band approximately 420 and 100 bp, the fourth one

was in *C. albicans,* and *C. tropicalis* with band approximately 400 bp and approximately 118 bp, the last pattern showed in *C. parapsilosis and C. krusei* with one band approximately 520 bp. Only the *Dde* I RFLP pattern of our study showed it can clearly identified *Candida* species as *C. glabrata, C. guilliermondii,* and *C. dubliniensis,* but difficult to identify in *C. albicans, C. tropicalis, C. parapsilosis* and *C. krusei.*

RFLP patterns of *Tru9* I in *Candida* species reference strains showed 7 types of RFLP patterns; *C. glabrata* show 5 bands approximately 250 bp, 230 bp, 120 bp, 90 bp and 50 bp, *C. guilliermondii* has 3 band approximately 400 bp, 90 and 40 bp. All of *C. dubliniensis*, *C. tropicalis*, and *C. parapsilosis* show 3 band-patterns of RFLP following, the band approximately 350 bp, 118 bp, and 40 bp for *C. dubliniensis,* the band approximately 350 bp, 100 bp, and 40 bp for C. *tropicalis*, and the band approximately 330 bp, 90 bp, and 50 bp for *C. parapsilosis.* The two bands of RFLP approximately 500 bp, and 40 bp was identified as *C. albicans*. The single band pattern approximately 500 bp of *Tru*9 I RFLP was found in *C. krusei.*

In our study of PCR-RFLP in reference strains, PCR products size could be identified two species of *Candida* (*C. glabrata,* and *C. guilliermondii*). *C. glabrata, C. guilliermondii, C. parapsilosis* and *C. krusei* were identified in RFLP-*Hae* III patterns. In the pattern of *Dde* I could be speciated three of *Candida* species: *C. glabrata, C. guilliermondii* and *C. dubliniensis.* The RFLP pattern of *Tru9* I could be identified seven species of *Candida* reference strain such as *C. glabrata, C. guilliermondii, C. parapsilosis,* C. *tropicalis*, *C. dubliniensis, C. krusei* and *C. albicans*. The combination results between *Hae* III and *Dde* I could be identified 5 species as *C. glabrata, C. guilliermondii, C. parapsilosis, C. krusei* and *C. dubliniensis.* All species were identified when used combination of *Tru9* I and *Hae* III or *Tru*9 I and *Dde* I or *Tru*9 I, *Dde* I and *Hae* III. All seven reference *Candida* spp. gave one pattern on *Hae* III, *Dde* I and *Tru9* I.

Figure 13. PCR amplification of *Candida* rDNA with ITS1 and ITS4 primers in seven difference *Candida* species reference strains

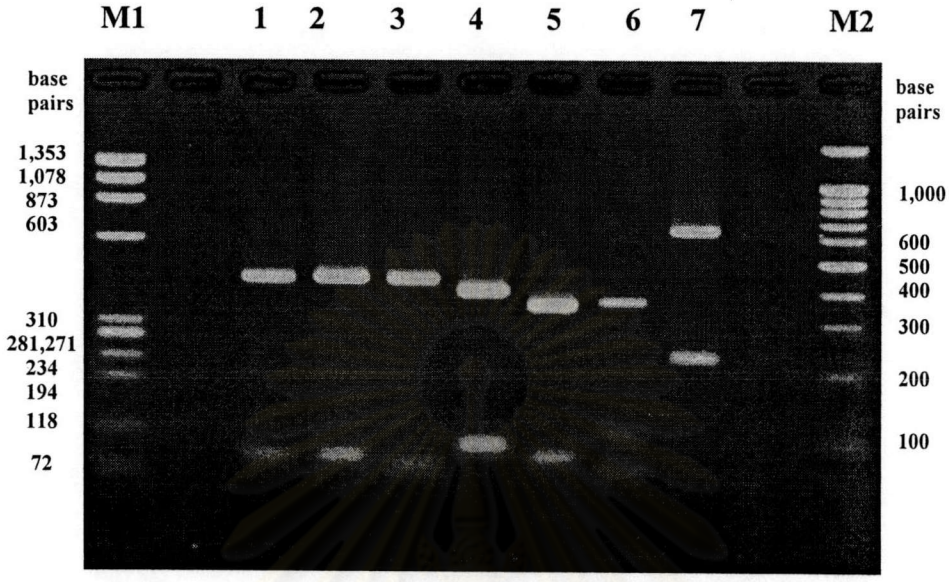


M1=Φx174/*Hae* III, 1= *C. albicans*, 2= *C. dubliniensis*, 3=*C. tropicalis*, 4= *C. parapsilosis*, 5= *C. krusei*, 6= *C. guilliermondii*, 7= *C. glabrata*, 8= Negative control, M2= 100bp ladder

Table 19. PCR products of *Candida* rDNA with ITS1 and ITS4 primers in seven difference *Candida* species reference strains

Organisms	GenBank Accession no.	PCR Products (base pairs)
C. albicans	AF217609	536
C. dubliniensis	AF321539	541
C. tropicalis	AF218966	523
C. parapsilosis	L47109	521
C. krusei	L47113	510
C. guilliermondii	AF405231	608
C. glabrata	AY168784	875

Figure 14. Restriction digestion of PCR products with the enzyme *Hae* III in seven difference *Candida* species reference strains

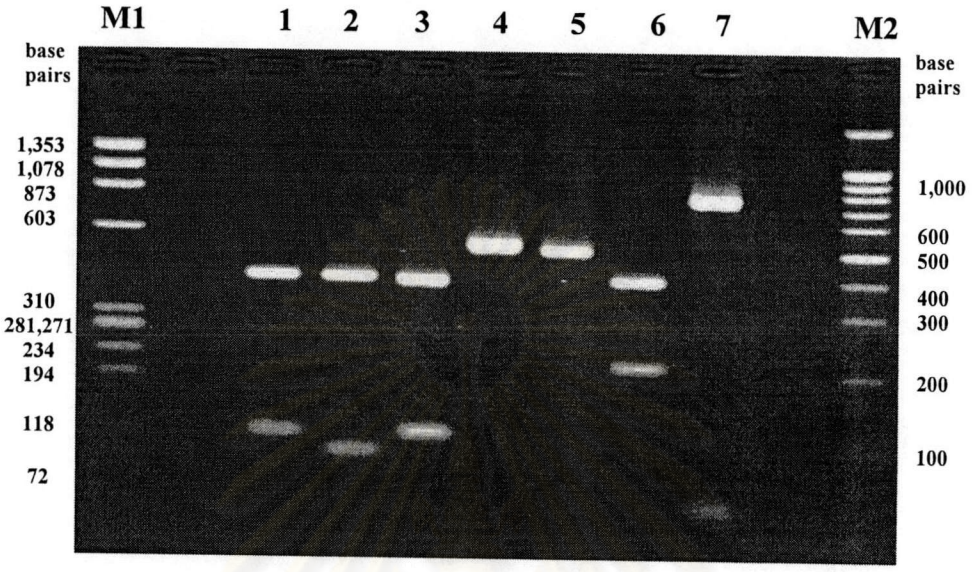


M1=Φx174/*Hae* III, 1= *C. albicans*, 2= *C. dubliniensis*, 3=*C. tropicalis*, 4= *C. parapsilosis*, 5= *C. krusei*, 6= *C. guilliermondii*, 7= *C. glabrata*, M2= 100bp ladder

Table 20. RFLP patterns of seven difference *Candida* species using *Hae* III enzyme analyzed by Bioedit program

Organisms	GenBank Accession no.	RFLP patterns of *Hae* III enzyme (base pairs)
C. albicans	AF217609	445,91
C. dubliniensis	AF321539	451,90
C. tropicalis	AF218966	445,78
C. parapsilosis	L47109	402,105
C. krusei	L47113	382,90
C. guilliermondii	AB105435	390, 117, 79
C. glabrata	AY168784	652,223

Figure 15. Restriction digestion of PCR products with the enzyme *Dde* I in seven difference *Candida* species reference strains

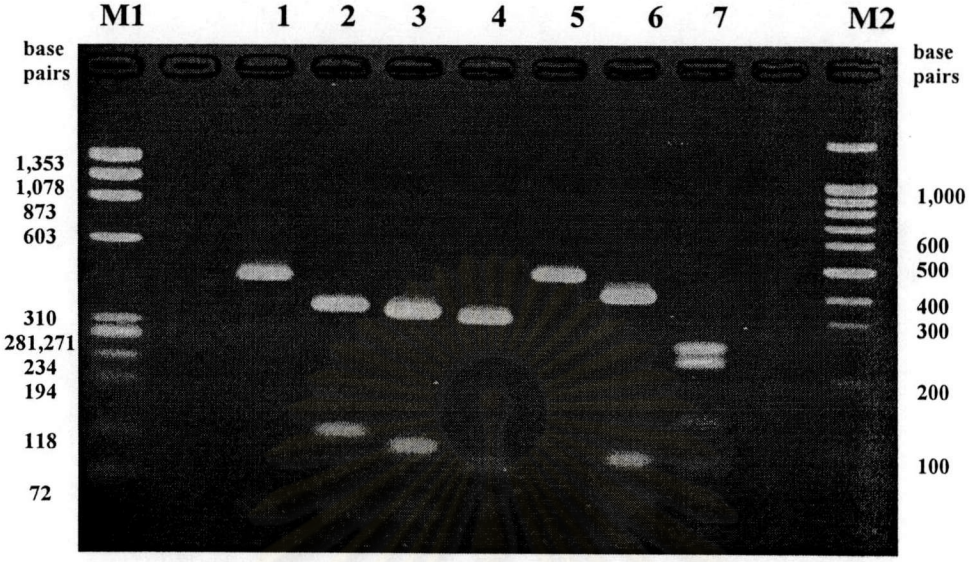


M1=Φx174/*Hae* III, 1= *C. albicans*, 2= *C. dubliniensis*, 3=*C. tropicalis*, 4= *C. parapsilosis*, 5= *C. krusei*, 6= *C. guilliermondii*, 7= *C. glabrata*, M2= 100bp ladder

Table 21. RFLP patterns of seven difference *Candida* species using *Dde* I enzyme analyzed by Bioedit program

Organisms	GenBank Accession no.	RFLP patterns of *Dde* III enzyme (base pairs)
C. albicans	AF217609	418,118
C. dubliniensis	AF321539	420,98
C. tropicalis	AF218966	410,113
C. parapsilosis	L47109	521
C. krusei	L47113	510
C. guilliermondii	AF405231	395,213
C. glabrata	AY168784	782,49,44

Figure 16. Restriction digestion of PCR products with the enzyme *Tru9* I in seven difference *Candida* species reference strains.



M1=Φx174/*Hae* III, 1= *C. albicans*, 2= *C. dubliniensis*, 3=*C. tropicalis*, 4= *C. parapsilosis*, 5= *C. krusei*, 6= *C. guilliermondii*, 7= *C. glabrata*, M2= 100bp ladder

Table 22. RFLP patterns of seven difference *Candida* species using *Tru9* I enzyme analyzed by Bioedit program

Organisms	**GenBank Accession no.**	**RFLP patterns of *Tru9* I enzyme** (base pairs)
C. albicans	AF217609	473,40
C. dubliniensis	AF321539	359,119,40
C. tropicalis	AF218966	344,101,40
C. parapsilosis	L47109	327, 85, 46
C. krusei	L47113	487
C. guilliermondii	AF405231	395,92,51
C. glabrata	AY168784	253,231,122,87,56,40

3.2 PCR products and RFLP patterns of *Candida* species from clinical specimen

One hundred and twenty PCR products from clinical isolates using ITS1 and ITS4 primers were analyzed with three restriction enzymes. Figure 17, 18, 19 and 20 show a typical gel electrophoresis of PCR products obtained from seven clinical isolated *Candida* species and digested with *Hae* III, *Dde* I, and *Tru9* I restriction enzymes, respectively, and the restriction profiles obtained with each enzyme for each isolate is summarized in Table 23-26. From the PCR analysis, there were 9 isolates that obtained PCR products size at approximately 800 bp (Fig 17; P5) and PCR products size approximately 600 bp were found 3 isolates (Fig 17; P4). Other remaining isolates showed approximately 500 bp of PCR product size (Fig 17; P1, P2, P3, P6 and P7).

The RFLP patterns of *Hae* III enzyme in this study found 9 isolates that obtained approximately 650 bp, and 220 bp of DNA fragments (Fig 18; H5), 3 isolates showed approximately 400 bp, 118 bp, 80 bp (Fig 18; H4), 19 isolates have approximately 400 bp, and 100 bp (Fig 18; H6), 2 isolates were approximately 380 bp, 90 bp, and 40 bp (Fig 18; H1), and 87 isolates with band at approximately 450 bp, and 90 bp (Fig 18; H2, H3, H7).

In *Dde* I patterns showed fragment at approximately 780 bp, and 50 bp was found in 9 isolates (Fig 19; D5), approximately 380 bp, and 210 bp was 3 isolates (Fig 19; D4), and approximately 420 bp and, 100 bp was 6 isolates (Fig 23; D3), approximately 400 bp, and 118 bp was 81 isolates (Fig 19; D2 and D7), and one band approximately 500 bp was 21 isolates (Fig 19; D1 and D6).

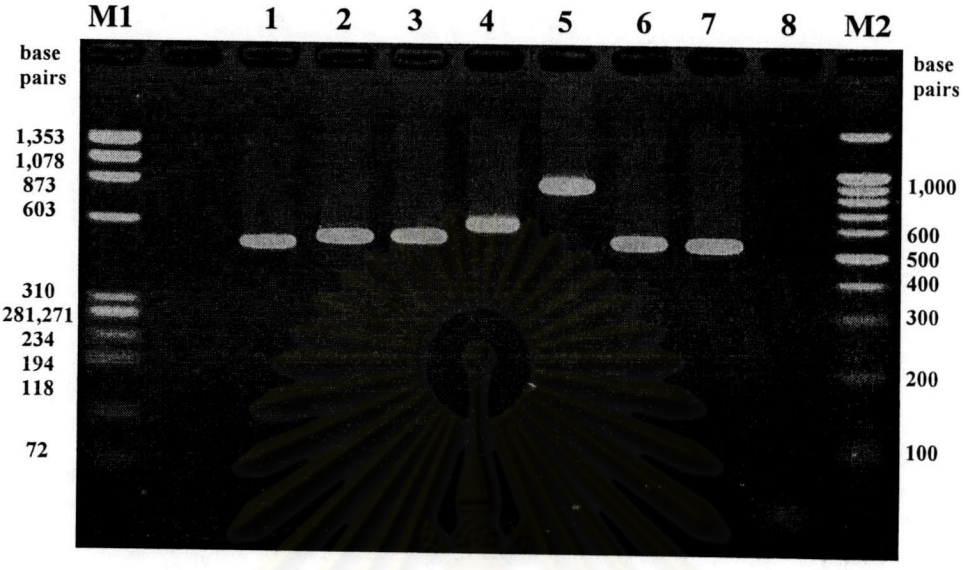
From RFLP patterns of *Tru9* I were showed 9 isolates with pattern of approximately 250 bp, 230 bp, 120 bp, 90 bp and 50 bp (Fig 20; T5), 3 isolates that band approximately 400 bp, 90 bp, and 40 bp (Fig 20; T4), 16 isolates that band approximately 350 bp, 118 bp, and 40 bp (Fig 20; T3 and Fig 25; CA5), 26 isolates with band approximately 350 bp, 100 bp, and 40 bp (Fig 20; T7 and Fig 25; CA6), 19 isolates with band approximately 330 bp, 100 bp and 40 bp (Fig 20; T6), 15 isolates with two bands approximately 480 bp, and 40 bp (Fig. 20; T2), 2 isolates with a single band approximately 490 bp (Fig 20; T1), 16 isolates with approximately 480 bp, 350 bp, 118 bp, and 40 bp (Fig 21; CA2), 3 isolates approximately 480 bp, 380 bp, 100 bp and 40 bp (Fig. 21; CA3), 7 isolates approximately 480 bp, 350 bp, 100 bp, and 40 bp

(Fig. 21; CA4), 2 isolates with band approximately 380 bp, 100 bp, and 40 bp (Fig. 21; CA7).

There are forty isolates, 16 isolates of CA2 pattern, 3 isolates of CA3, 7 isolates of CA4, 10 isolates of CA5, 2 isolates of each CA6 and CA7, that has atypical patterns from *Candida* species reference strains in RFLP of *Tru9* I enzyme whereas conventional assay showed these strains were *C. albicans,* also the PCR products and RFLP profiles of *Hae* III and *Dde* I showed correspond pattern to *C. albicans* reference strain (Fig 22). The *Tru9* I atypical patterns were shown in Fig 21 (CA2 to CA7). Then, the DNA of forty atypical *Tru9* I patterns were confirmed with *Mbo* I, the results showed that profiles of RFLP correspond to *C. albicans* reference strain. The fragment of DNA in cutting with *Mbo* I was approximately 200 bp, and two bands at approximately 160 and 140 bp (Fig 23). Table 27 showed the number of isolates and summarized RFLP patterns of *C. albicans* atypical *Tru9* I profiles. As describe above in reference strains, *Hae* III and *Dde* I could identify the *Candida* spp. same as reference strains but *Tru9* I could not identify some of *C. albicans* (Table 28).

From 120 clinical isolates were analysis by PCR-RFLP patterns of three enzymes (*Hae* III, *Dde* I and *Tru9* I) and some isolates (40 of *Tru9* I atypical patterns isolates) confirmed with *Mbo* I that compared with *Candida* reference strains patterns showed, 55 (45.8%) *C. albicans*, 26 (21.7%) *C. tropicalis*, 19 (15.8%) *C. parapsilosis,* 9 (7.5%) *C. glabrata,* 6 (5.0%) *C. dubliniensis,* 3 (2.5%) *C. guilliermondii,* and 2 (1.7%) *C. krusei*, data shown in Table 29 and Figure 24. All *Candida* species from clinical isolate except *C. albicans* gave the patterns same as the reference strain patterns in the same species. *C. albicans* gave the same pattern with *C. albicans* reference strain in *Hae* III and *Dde* I but not in *Tru* 9 I. There are 7 patterns of *C. albicans* RFLP in cutting with *Tru9* I, one pattern is same as the reference strain and other six are difference from reference strains.

Figure 17. PCR amplification of *Candida* rDNA with ITS1 and ITS4 primers in clinical isolates.

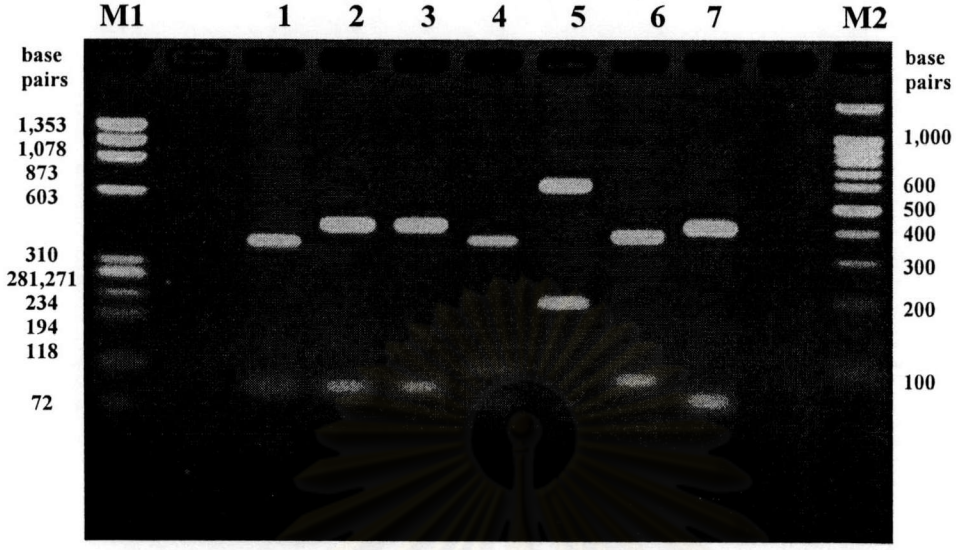


M1=Φx174/*Hae* III , 1= P1, 2= P2, 3= P3,4= P4, 5= P5 , 6= P6, 7= P7, 8= Negative control, M2= 100bp ladder

Table 23. PCR patterns of *Candida* species in clinical specimens using ITS1 and ITS4 primers

Pattern ID	Lane no.	Strain no.	PCR Products (base pairs)	Expected *Candida* species
P1	1	IC-14	500	?
P2	2	IC-16	500	?
P3	3	IC-23	500	?
P4	4	IC-53	600	*C. guilliermondii*
P5	5	IC-76	800	*C. glabrata*
P6	6	IC-84	500	?
P7	7	IC-90	500	?

Figure 18. Restriction digestion of PCR products with the enzyme *Hae* III in clinical isolates

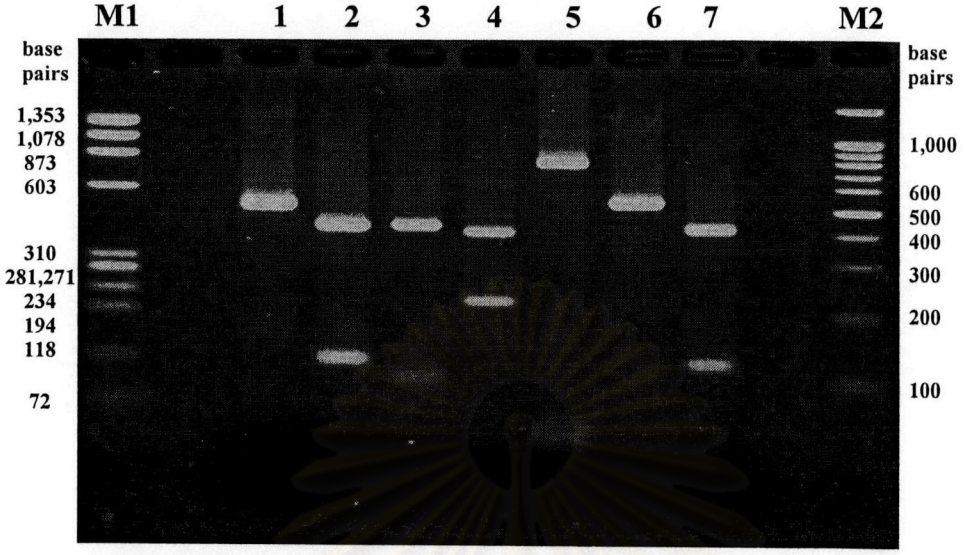


M1=Φx174/*Hae* III , 1= H1, 2= H2, 3= H3,4= H4, 5= H5 , 6= H6, 7= H7, M2= 100bp ladder

Table 24. RFLP patterns of *Candida* species in clinical specimens using *Hae* III enzyme

Pattern ID	Lane no.	Strain no.	RFLP patterns of *Hae* III enzyme (base pairs)	Expected *Candida* species
H1	1	IC-14	380, 90, 40	*C. krusei*
H2	2	IC-16	450,90	?
H3	3	IC-23	450,90	?
H4	4	IC-53	400,118, 80	*C. guilliermondii*
H5	5	IC-76	650, 220	*C. glabrata*
H6	6	IC-84	400,100	*C. parapsilosis*
H7	7	IC-90	450,90	?

Figure 19. Restriction digestion of PCR products with the enzyme *Dde* I in clinical isolates

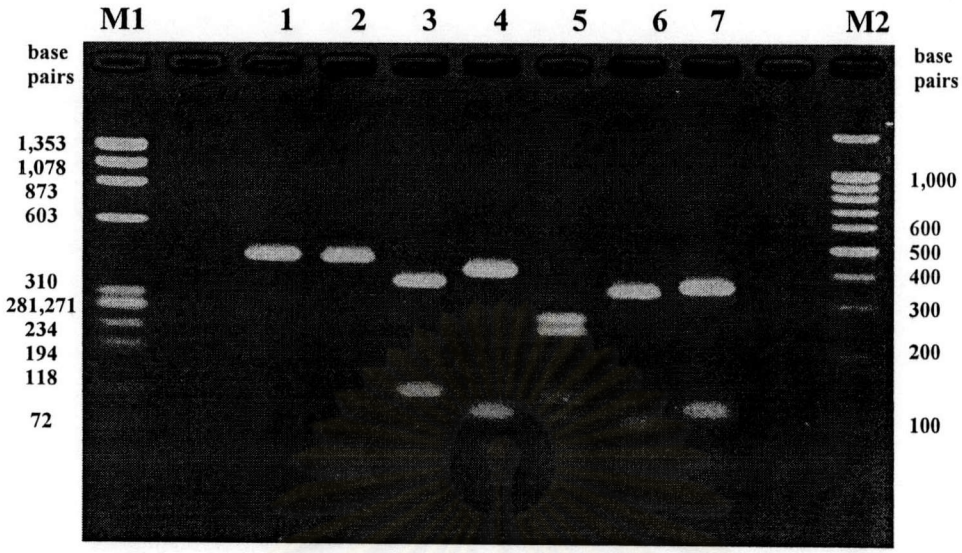


M1=Φx174/*Hae* III , 1= D1, 2= D2, 3= D3,4= D4, 5= D5 , 6= D6, 7= D7,M2= 100bp ladder

Table 25. RFLP patterns of *Candida* species in clinical specimens using *Dde* I enzyme

Pattern ID	Lane no.	Strain no.	RFLP patterns of *Dde* I enzyme (base pairs)	Expected *Candida* species
D1	1	IC-14	500	?
D2	2	IC-16	400,118	?
D3	3	IC-23	420, 100	*C. dubliniensis*
D4	4	IC-53	380, 210	*C. guilliermondii*
D5	5	IC-76	780, 50	*C. glabrata*
D6	6	IC-84	500	?
D7	7	IC-90	400, 118	?

Figure 20. Restriction digestion of PCR products with the enzyme *Tru9* I in clinical isolates

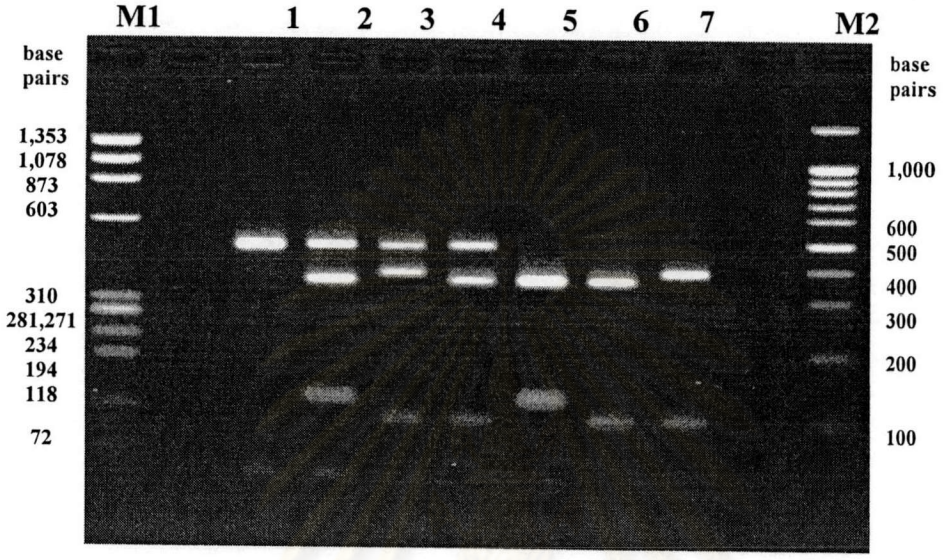


M1=Φx174/*Hae* III , 1= T1, 2= T2, 3= T3,4= T4, 5= T5 , 6= T6, 7= T7, M2= 100bp ladder

Table 26. RFLP patterns of *Candida* species in clinical specimens using *Tru9* I enzyme

Pattern ID	Lane No.	Strain No.	RFLP patterns of *Tru9* I enzyme (base pairs)	Expected *Candida* species
T1	1	IC-14	490	*C. krusei*
T2	2	IC-16	480, 40	*C. albicans*
T3	3	IC-23	350,118, 40	*C. dubliniensis*
T4	4	IC-53	400,90,40	*C. guilliermondii*
T5	5	IC-76	250, 230, 120, 90, 50	*C. glabrata*
T6	6	IC-84	330,100, 40	*C. parapsilosis*
T7	7	IC-90	350, 100, 40	*C. tropicalis*

Figure 21. RFLP patterns of *C. albicans* atypical *Tru9* I RFLP patterns.



M1=Φx174/*Hae* III

Lane 1 = *C. albicans* reference strain (CA1)

2 = IC-6 (CA2)

3 = IC-34 (CA3)

4= IC-42 (CA4)

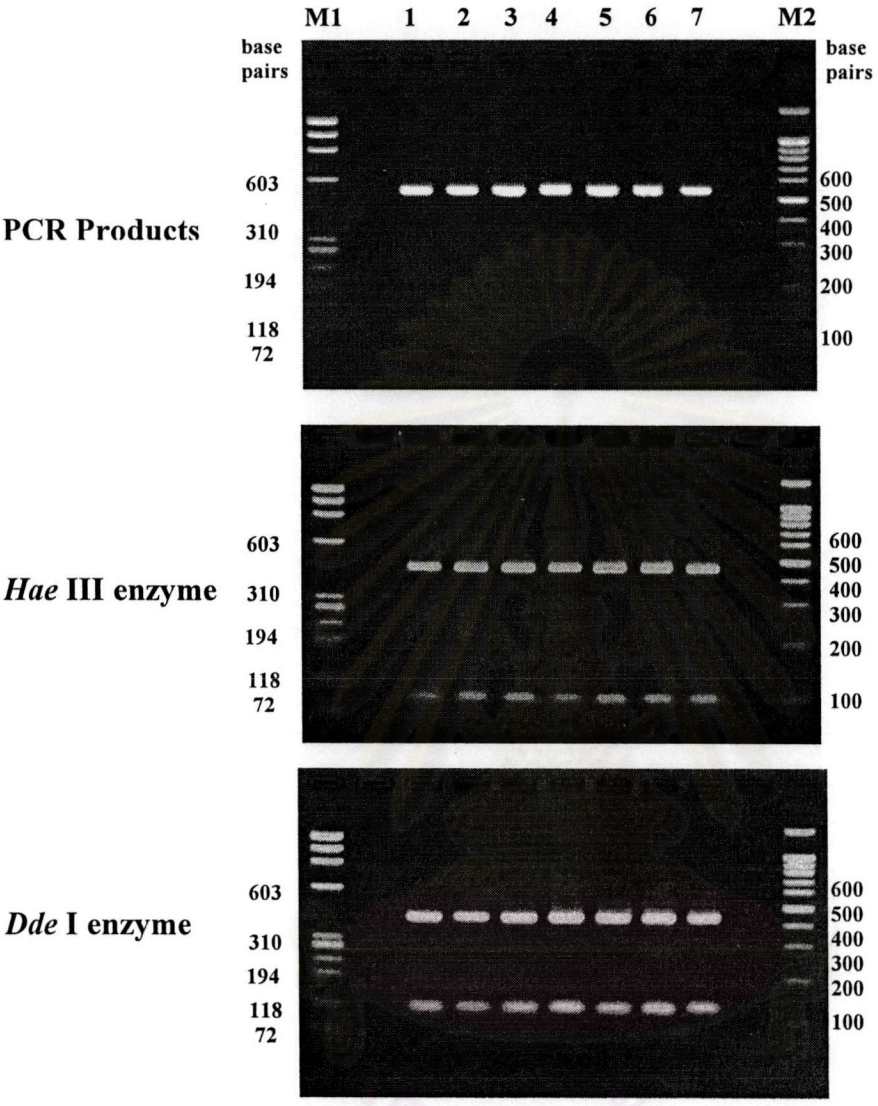
5= IC-63 (CA5)

6= IC-58 (CA6)

7= IC-115 (CA7)

M2= 100bp ladder

Figure 22. PCR products and RFLP patterns in *Hae* III and *Dde* I of *C. albicans* atypical *Tru9* I RFLP patterns



M1=Φx174/*Hae* III

Lane 1 = *C. albicans* reference strain (CA1)

2 = IC-6 (CA2)

3 = IC-34 (CA3)

4= IC-42 (CA4)

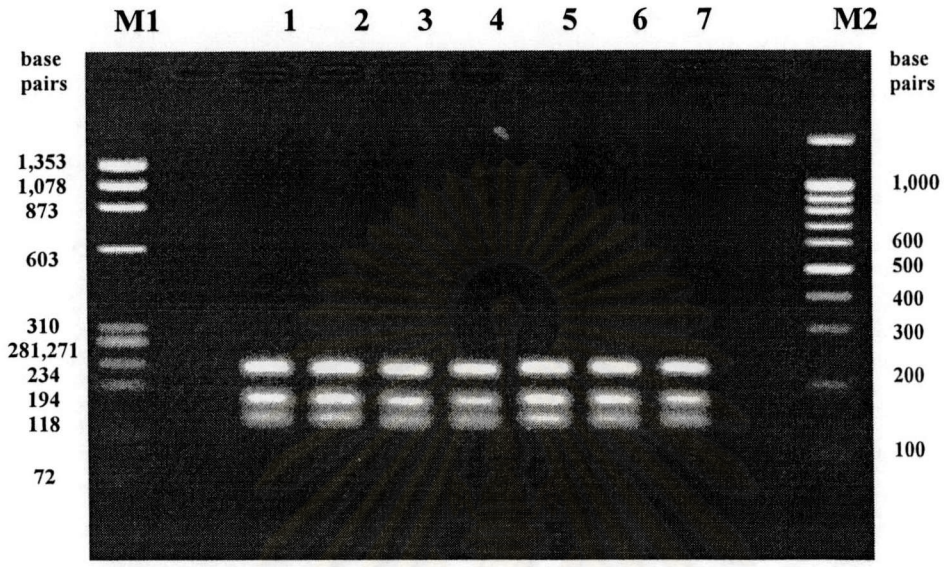
5= IC-63 (CA5)

6= IC-58 (CA6)

7= IC-115 (CA7)

M2= 100bp ladder

Figure 23. RFLP patterns in *Mbo* I of *C. albicans* atypical *Tru9* I RFLP patterns



M1=Φx174/*Hae* III Lane 1= CA1, 2= CA2 ,3= CA3, 4= CA4, 5= CA5, 6= CA6, 7= CA7, M2= 100bp ladder

Table 27. Summarize of RFLP atypical patterns of *C. albicans* in clinical specimens

Type No.	No. of isolates	Products size of PCR - RFLP				
		PCR	*Hae* III	*Dde* I	*Tru9* I	*Mbo* I
CA1	15	500	450,90	400, 118	480, 40	210, 160, 140
CA2	16	500	450,90	400,118	480, 350, 118,40	210, 160, 140
CA3	3	500	450,90	400, 118	480, 380,100,40	210, 160, 140
CA4	7	500	450,90	400, 118	480, 350, 100, 40	210, 160, 140
CA5	10	500	450,90	400, 118	350, 118, 40	210, 160, 140
CA6	2	500	450,90	400, 118	350, 100, 40	210, 160, 140
CA7	2	500	450,90	400, 118	380, 100,40	210, 160, 140

Table 28. Summarize *Candida* spp. and number of isolates from each enzyme

Enzyme	No. of identified isolate	No. of unidentified isolate	Identified *Candida* spp.
Hae III	33	87	I1, I2, I3, I4
Dde I	18	102	I2, I3, I5
Tru9 I*	80	40	I1, I2, I3, I4, I5, I6, I7
Hae III plus *Dde* I	39	40	I1, I2, I3, I4, I5
Hae III plus *Tru9* I*	80	40	I1, I2, I3, I4, I5, I6, I7
Dde I plus *Tru9* I*	80	40	I1, I2, I3, I4, I5, I6, I7
Dde I , *Hae* III and *Tru9* I*	80	40	I1, I2, I3, I4, I5, I6, I7
Tru 9 I plus *Mbo* I**	40	-	I7

* Only *C. albicans* that have the same RFLP patterns as reference strain.

** Assay only in atypical *Tru9* I patterns of *C. albicans*

I1=*C. krusei*, I2= *C. guilliermondii*, I3= *C. glabrata*, I4=*C. parapsilosis*,

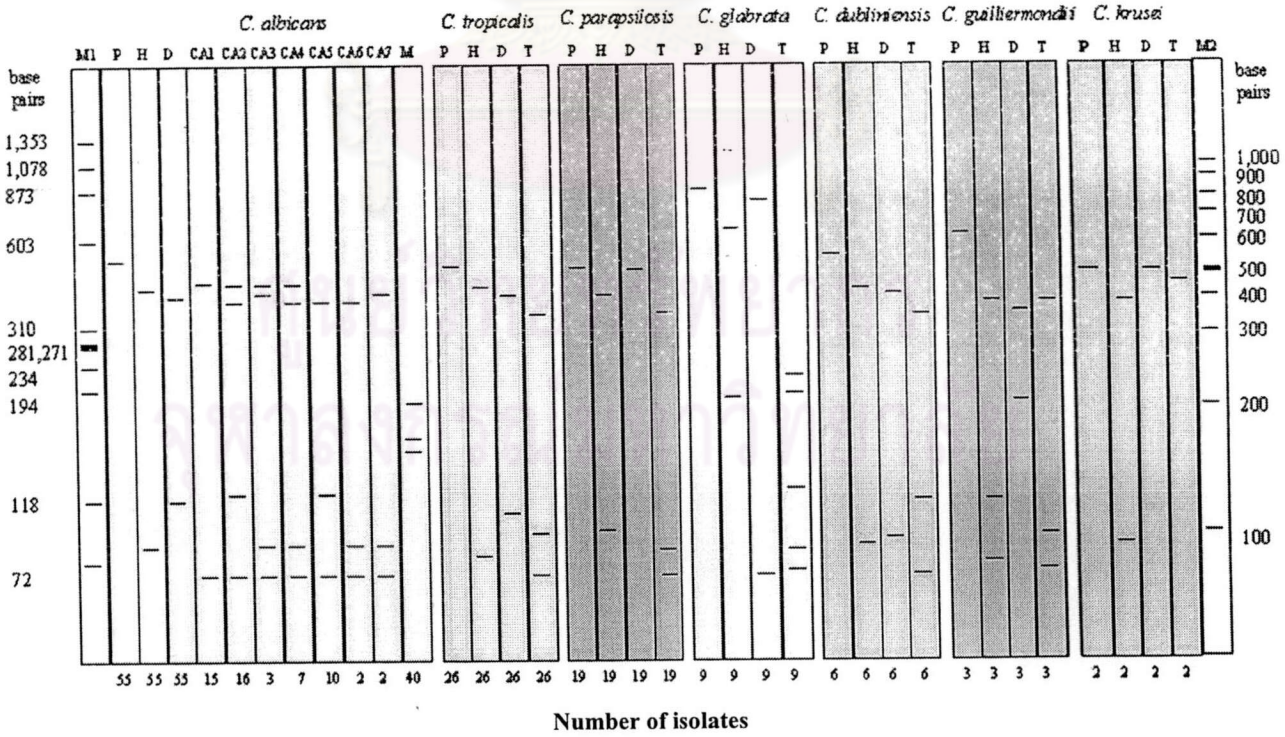
I5= *C. dubliniensis*, I6= *C. tropicalis*, I7 = *C. albicans*

Table 29. Summarize of *Candida* isolate in clinical specimens by PCR-RFLP

Candida species	No of isolates	Similarity Patterns with reference strains							
		Hae III		*Dde* I		*Tru9* I		*Mbo* I	
		Yes	No	Yes	No	Yes	No	Yes	No
C. albicans	55	55	0	55	0	15	40	40	0
C. tropicalis	26	26	0	26	0	26	0	n/a	n/a
C. parapsilosis	19	19	0	19	0	19	0	n/a	n/a
C. glabrata	9	9	0	9	0	9	0	n/a	n/a
C. dubliniensis	6	6	0	6	0	6	0	n/a	n/a
C. guilliermondii	3	3	0	3	0	3	0	n/a	n/a
C. krusei	2	2	0	2	0	2	0	n/a	n/a

n/a = not assay

Figure 24. Summarize of PCR-RFLP patterns of 120 *Candida* isolates in clinical specimens



M1=Φx174/*Hae* III, P = PCR, H = *Hae* III, D= *Dde* I, T= *Tru9* I, CA1= typical *Tru 9* I in *C. albicans* patterns, CA2-CA7 = atypical *Tru 9* I in *C. albicans* patterns, M= *Mbo* I M2=100 bp ladder

3.4 Comparison between a conventional method plus *Candida* commercial kits (API 20C AUX) and PCR RFLP results

The comparative results of conventional and PCR-RFLP were showed in Table 30. The result of conventional method plus API 20C AUX and PCR-RFLP were correctly in identification of five *Candida* species; 26 *C. tropicalis*, 19 *C. parapsilosis*, 9 *C. glabrata*, 3 *C. guilliermondii*, and *2 C. krusei*. In sixty-one isolates of *C. albicans* that resulted from conventional plus API showed correctly only 55 isolates with PCR-RFLP, whereas 6 isolates showed as *C. dubliniensis* by PCR-RFLP.

Table 30. Comparison results between conventional method plus *Candida* commercial kits (API 20C AUX) and PCR-RFLP assay

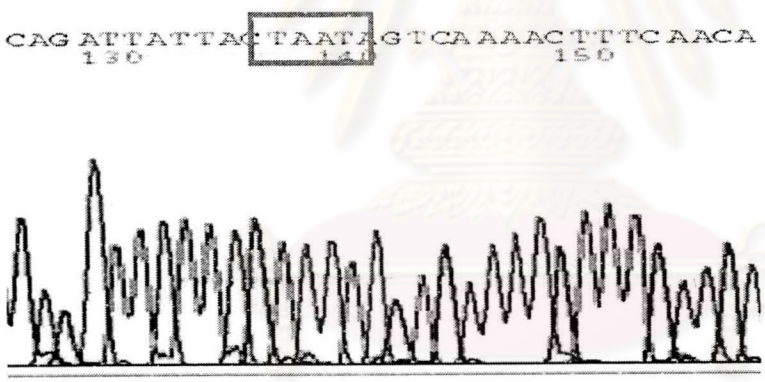
PCR-RFLP / Conventional and API	PCR-RFLP Assay							
	C. albicans	*C. tropicalis*	*C. parapsilosis*	*C. glabrata*	*C. dubliniensis*	*C. guilliermondii*	*C. krusei*	Total
C. albicans	55				6			61 (50.8%)
C. tropicalis		26						26 (21.7%)
C. parapsilosis			19					19 (15.8%)
C. glabrata				9				9 (7.5%)
C. dubliniensis					0			0 (0%)
C. guilliermondii						3		3 (2.5%)
C. krusei							2	2 (1.7%)
Total	55 (45.8%)	26 (21.7%)	19 (15.8%)	9 (7.5%)	6 (5.0%)	3 (2.5%)	2 (1.7%)	120 (100%)

3.5 Sequencing of *C. albicans Tru9* I atypical patterns compared with *C. albicans* reference strain.

One of six *C. albicans Tru9* I atypical patterns isolates (Fig 21; CA2) was analyzed by sequencing analysis compared with the *C. albicans* reference strain (Fig 21; CA1). This strain showed the insertion mutation described at position 140 of rDNA in ITS region using ITS1 and ITS4 primers (Fig 25B). Figure 25A showed a sequence of reference strain at the same position.

Figure 25. The chromatogram obtained from automate sequencing showed insertion mutation within ITS region of *C. albicans* rDNA, A) showed the reference strain and B) showed the clinical strain

A)



B)