RESULTS

Identification

On the basis of chlamydospore production and germ tube formation, forty-two strains out of two hundred isolates of C. albicans were obtained. Only twenty-three strains were selected to test sugar fermentations and protein pattern by disc electrophoresis.

All of twenty-three strains showed sugar fermentation pattern conformed to the classical taxonomic descriptions (Lodder, 1952 and Rippon, 1974), which all of them utilized glucose, maltose and produced acid and gas. Usually C. albicans could not use lactose as a carbon source. There were some variations of sucrose utilization. The reaction varied from active to inactive fermentation as shown in Table I. Seven strains of sputum isolates, one strain from urine and one strain from unknown No. 2 gave active reaction on sucrose fermentation. Eight strains isolated from vagina showed slightly and more slightly fermentation of sucrose in equal numbers of isolates. There were two strains showed negative reaction on sucrose fermentation.

Protein Preparation and Disc Electrophoresis

The first method had been used for protein preparation was followed Shechter et. al. (1972). The yeast cells

No	Source of isolates	Sugar Fermentation			
		Glucose	Maltose	Lactose	Sucrose
1	Sputum	AG	AG	O	A
2	Sputum	AG	AG	O	A
3	Sputum	AG	AG	O	A
4	Sputum	AG	AG	O	A
5	Sputum	AG	AG	O	A
6	Sputum	AG	AG	O	A
7	Sputum	AG	AG	O	A
8	Urine	AG	AG	O	A
9	Unknown No.2	AG	AG	O	A
10	Vagina	AG	AG	O	Slightly
11	Vagina	AG	AG	O	Slightly
12	Vagina	AG	AG	O	Slightly
13	Vagina	AG	AG	O	Slightly
14	Mouth	AG	AG	O	Slightly
15	Skin No. 2	AG	AG	O	Slightly
16	Vagina	AG	AG	O	More slightly
17	Vagina	AG	AG	O	More slightly
18	Vagina	AG	AG	O	More slightly
19	Vagina	AG	AG	O	More slightly
20	Ear	AG	AG	O	More slightly
21	Unknown No.1	AG	AG	O	More slightly
22	Vagina	AG	AG	O	O
23	Skin No. 1	AG	AG	O	O

Table I. The sugar fermentation patterns of twenty-three strains of C. albicans from different lesions.
A = Acid; G = Gas; O = no reaction

were disrupted in cold buffer with acid-washed sand in a mechanical homogenizer (Sorvall, Omni-Mixer). The protein was determined by Lowry's method (1951), the amount was quite low and was not enough for loading on gel electrophoresis.

Another method, Gunsalus' technique (1955), was decided to use for protein preparation. He recommended that freshly prepared acetone dried cells may also yield active enzyme extraction on stirring with 0.004 M $NaHCO_3$ as suitable buffer which was also chosen in this study. The amount of protein yield by this method was high enough for loading the protein on disc gel electrophoresis.

The amount of protein average per 100 mg. of dried acetone powder varied from 200-300 ug. in different isolates. A large amount approximately 200-300 mg. of acetone powder was utilized in order to get the desired amount of soluble proteins for loading on gel electrophoresis.

The electrophoretic Rf value of water soluble proteins extract from yeast cells of three-day-old cultures in Sabouraud medium of twenty-three strains human pathogens, Candida albicans, other two species, C. krusei and C. utilis were detected and shown in Table 2.

Table 2. Average Rf values X 10 of soluble protein bands by disc electrophoresis of twenty-three strains of pathogens, C. albicans, and two strains of C. krusei, C. utilis saprobe.

No. of isolation \ Rf values X 10	0	1	2	3	4	5	6	7	8	9
vagina 1	-	1.37	2.83	3.71	4.37	5.37	6.37	-	8.61	9.50
							6.87			
2	-	1.57	2.81	3.71	4.38	5.06	6.07	-	8.65	9.55
							6.63			
3	-	1.58	2.79	3.41	4.39	5.24	6.22	-	8.9	9.51
							6.83			
4	-	1.46	2.77	3.37	4.39	5.24	6.09	-	8.79	9.51
							6.70			
11	-	1.49	2.76	3.39	4.48	5.28	6.20	-	-	9.02
				3.79			6.89			9.65
12	-	1.79	2.86	3.56	4.71	5.61	6.29	-	-	9.22
				3.81			6.96			9.66

Table 2. (Cont'd.)

No. of isolates \ Rf values X 10	0	1	2	3	4	5	6	7	8	9
vagina 13	-	1.06	2.67	3.86	4.64	5.35	6.47	7.60	8.13	9.20
		1.55					6.90			9.72
14	-	1.04	2.92	3.47	4.65	5.34	6.57	7.12	8.19	9.34
		1.91		3.96				7.64		9.72
29	-	1.58	2.80	3.51	4.14	5.61	6.34	-	8.31	9.51
					4.75					
sputum										
5	-	1.44	2.65	3.49	4.34	5.18	6.26	-	8.91	9.52
							6.74			
6	-	1.43	2.62	3.63	4.46	5.23	6.19	-	-	9.05
							6.90			9.64
7	-	1.44	2.64	3.71	4.67	5.44	6.33	7.00	8.93	9.55
8	-	1.43	2.66	3.96	4.28	5.12	6.19	-	8.90	9.64
							6.78			
9	-	1.48	2.86	3.69	4.44	5.06	6.08	-	-	9.05
							6.67			9.63
15	-	1.11	2.63	3.96	4.65	5.21	6.48	7.04	8.05	9.30
		1.55	2.92					7.57		9.72

Table 2. (Cont'd.)

No. of isolates / Rf values X 10		0	1	2	3	4	5	6	7	8	9
sputum	23	-	1.06	2.82	3.94	4.58	5.14	6.53	7.08	8.17	9.72
			1.80						7.74	8.73	
urine	10	0.91	1.65	2.59	3.56	4.23	5.00	6.39	-	8.16	9.25
					3.91		5.73			8.73	9.52
ear	17	-	1.94	2.83	3.86	4.58	5.42	6.25	-	8.99	9.58
								6.81			
mouth	31	-	1.44	2.75	3.73	4.46	5.06	6.14	-	8.72	9.65
								6.87			
unknown	33	-	1.64	2.86	3.76	4.43	5.19	6.20	-	8.87	9.62
								6.83			
skin	34	-	1.58	2.73	3.69	4.27	5.00	6.09	-	8.61	9.51
								6.71			
unknown	35	0.54	1.67	2.26	3.87	4.52	5.12	6.30	-	-	9.88
							5.76	6.90			
skin	42	-	1.34	2.65	3.61	4.39	5.00	6.22	7.47	-	9.34
								6.83			9.63
C. krusei		0.26	1.45	2.21	3.64	4.60	5.26	6.58	7.37	-	9.74
				2.60							
C. utilis		-	-	-	-	4.59	5.29	6.21	7.09	-	9.53
									7.90		

The migration distance from the origin to the front of each extracted proteins were recorded and used for calculation of Rf values. The calculation was based on the migration distance of bands divided by the distance of front and multiplied by 10. The densitometer was used on the purpose of detected the location of protein bands in gel column.

Average Rf values (Table 2) of each protein band in the electrophoretic patterns were compared among the intraspecies and interspecies to determine the number of the common bands which based on equal Rf values. The standard error of the bands which considered matching was ranged 0-0.198.

The protein bands of all twenty-five isolates were drawn based on the Rf values and shown in figure 12. The photograph of protein bands in the gel column was shown in figure 8.

The number of soluble protein bands of C. albicans varied from nine to thirteen bands as shown in figure 12. On the basis of infected locations, isolates of C. albicans were divided into three groups, vaginal group, sputum group and the group of various locations.

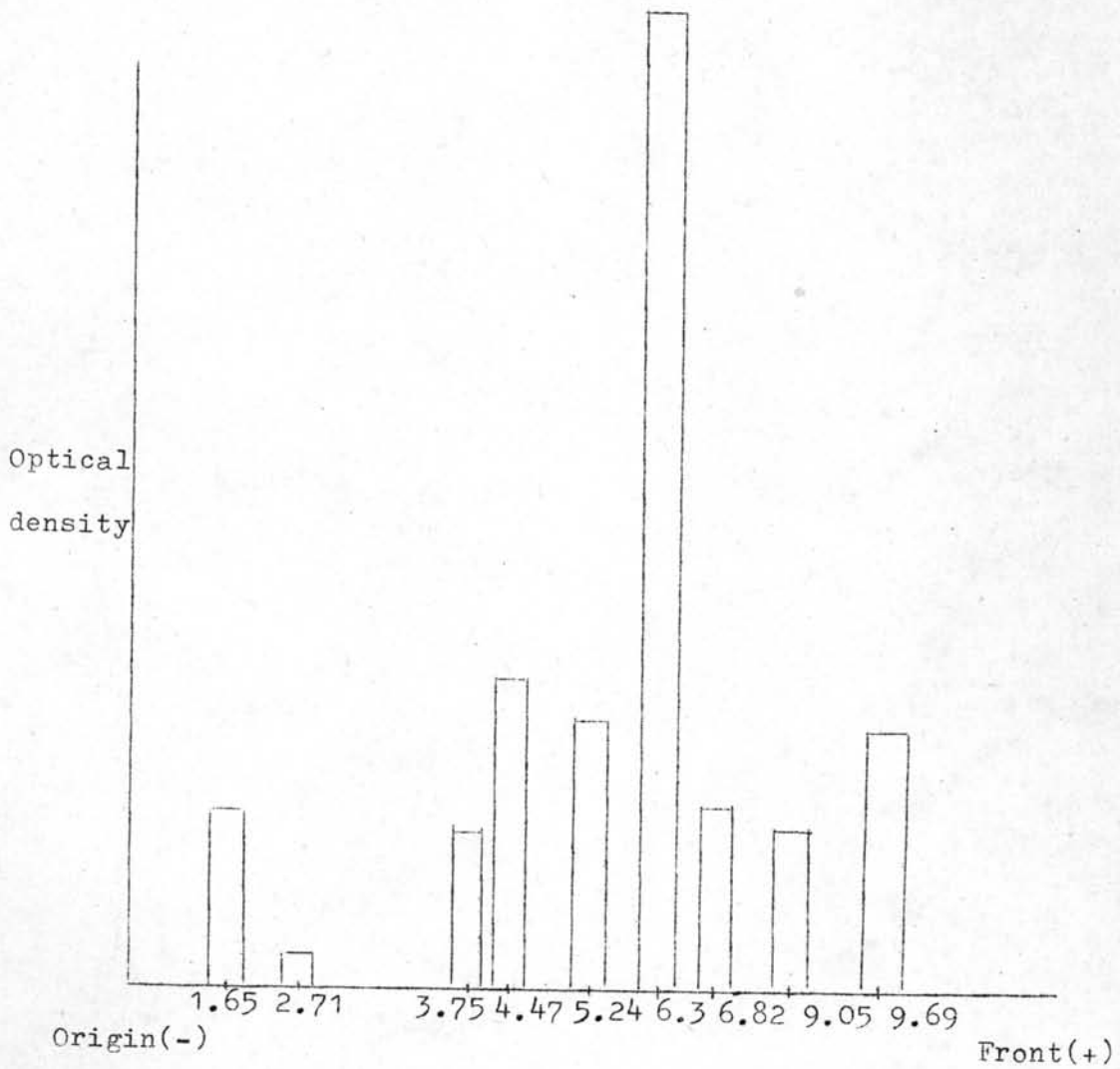

Figure. 7 Shown typical protein pattern of C. albicans which recorded by Densitometer.

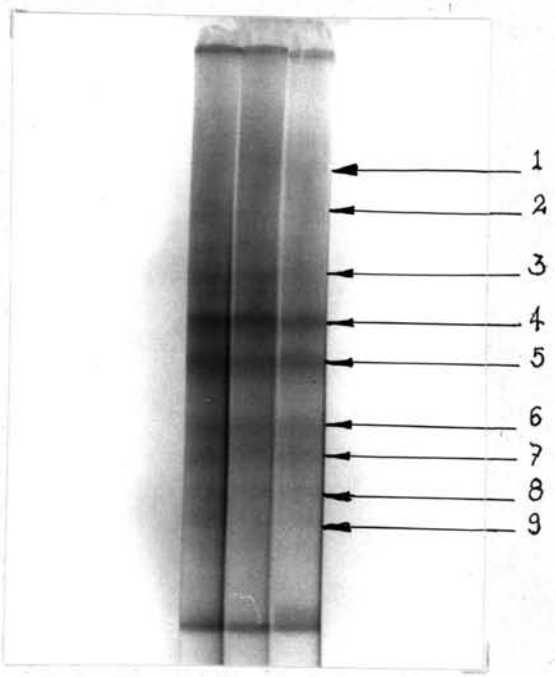

Figure 8 Photograph of electrophoretic acrylamide gel column of C. albicans staining with coomassie blue.

The electrophoretic patterns of vaginal strains were shown in figure 9. There were nine common bands at the average Rf values 1.65, 2.71, 3.75, 4.47, 5.24, 6.30, 6.82, 9.05, and 9.69 and there were four uncommon bands at Rf 1.08, 3.32, 7.47 and 8.12.

In figure 10, seven strains of sputum isolate showed nine common bands at Rf 1.65, 2.71, 3.75, 4.47, 5.24, 6.30, 6.82, 9.05 and 9.69 respectively. Only one strain showed 13 bands with four bands that did not match at Rf 1.08, 3.32, 7.47 and 8.12 and one strain showed 12 bands lacking of the band at the Rf 3.32,

In figure 11, there were seven strains which were isolated from various lesions. Nine bands were shown in common at Rf 1.65, 2.71, 3.75, 4.47, 5.24, 6.30, 6.82, 9.05 and 9.69 respectively. There were three strains shown some variations. The strains isolated from urine and strain unknown No.2 isolated from unknown location had only eight and seven matching bands the urine strain lacked the band at the Rf 6.82 and strain unknown No. 2 lacked the bands at the Rf 6.30 and 9.05.

The strains isolated from vagina No. 5,6 and 8 showed the band at the Rf 3.32 which had seen in the strain No. 6 of sputum strains. Two strains from vagina, No. 8 and No. 9,

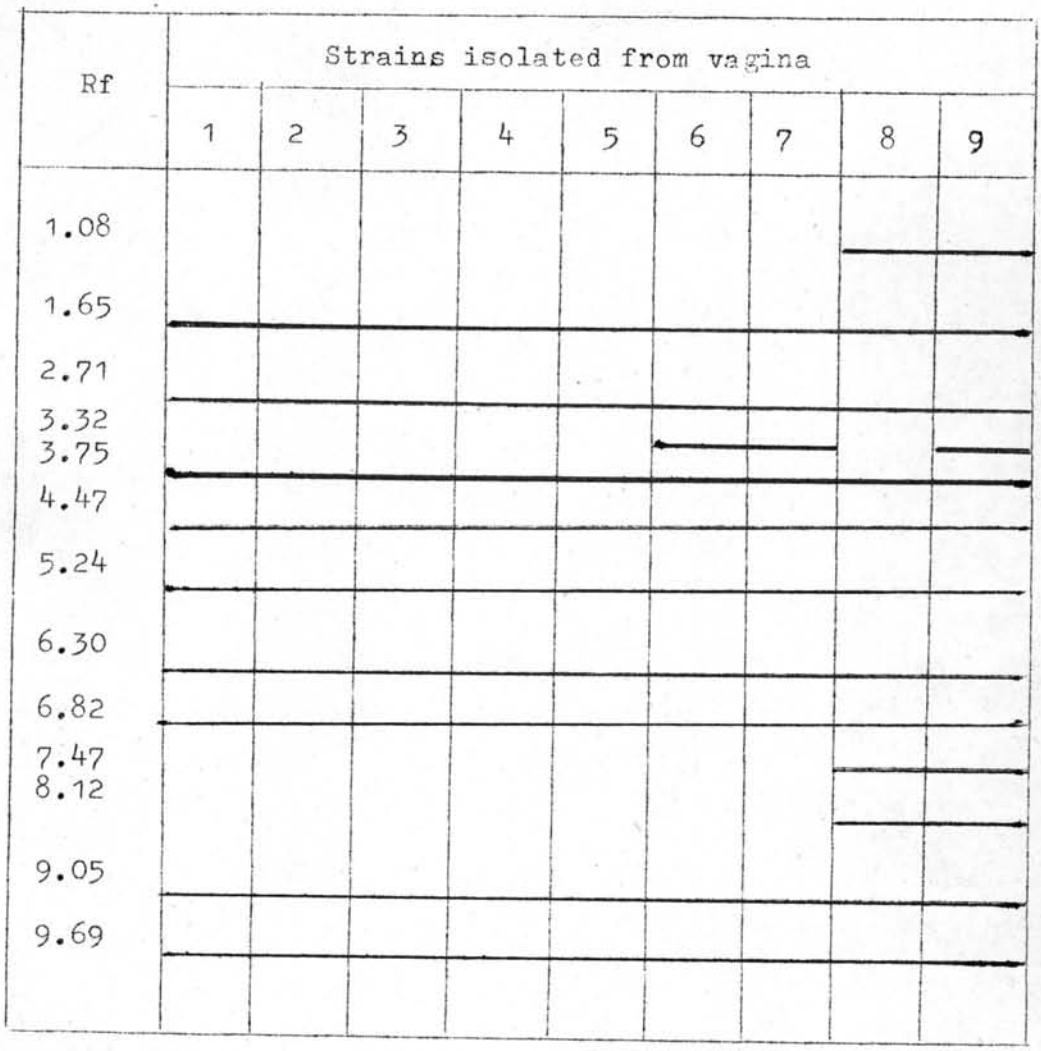

Figure 9 Diagram of acidic electrophoretic protein pattern of nine strains of C. albicans vaginal isolates.

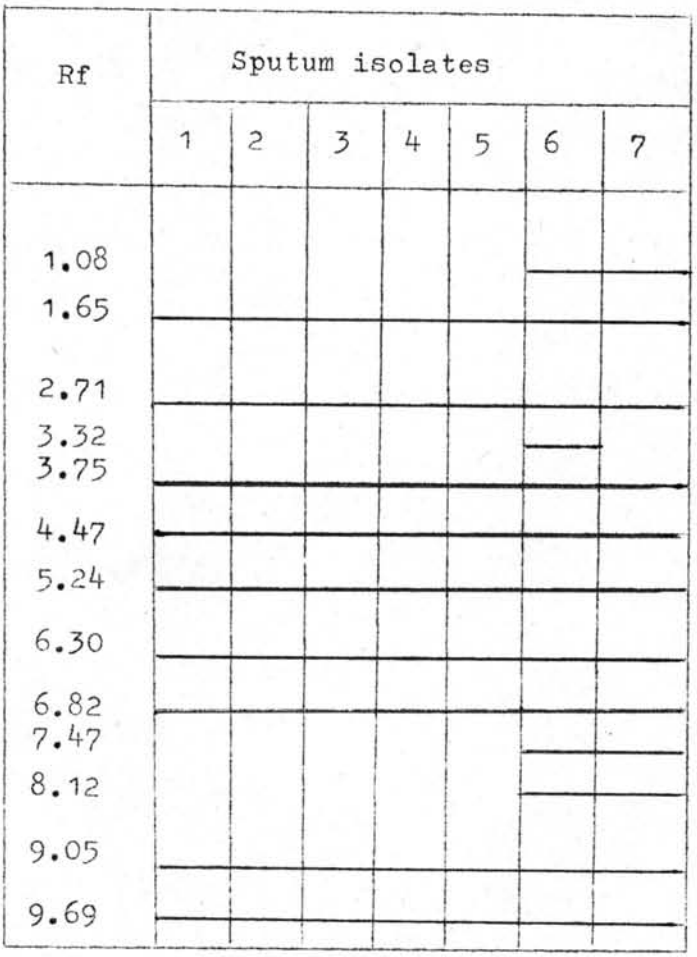

Figure 10 Diagram of acidic electrophoretic protein pattern of seven strains of C. albicans sputum isolates.

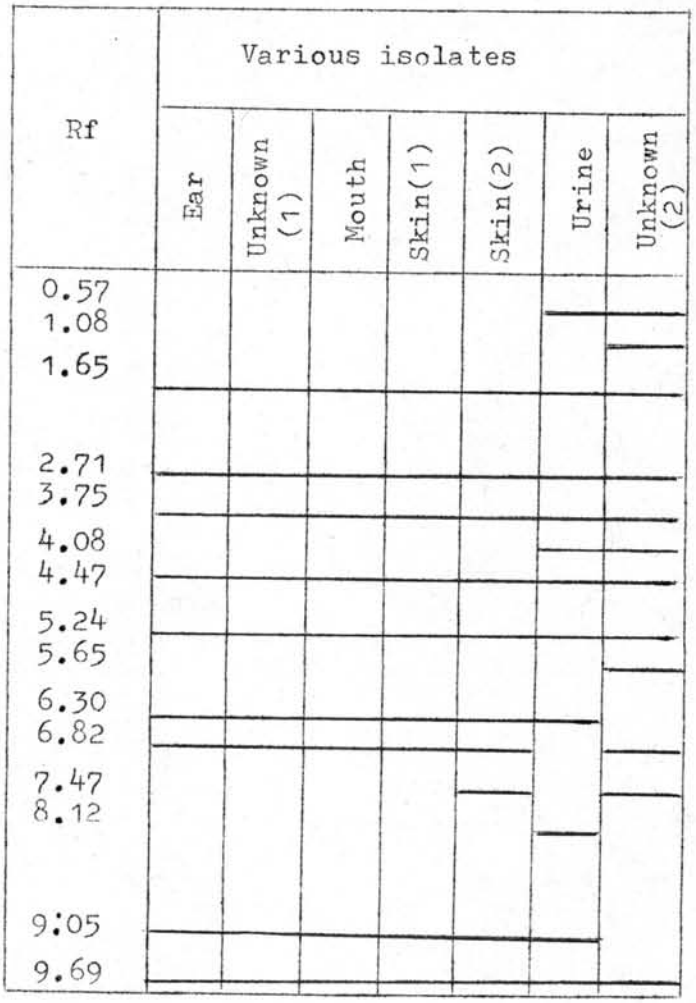

Figure 11 Diagram of acidic electrophoretic protein pattern of seven strains of C. albicans from various lesions.

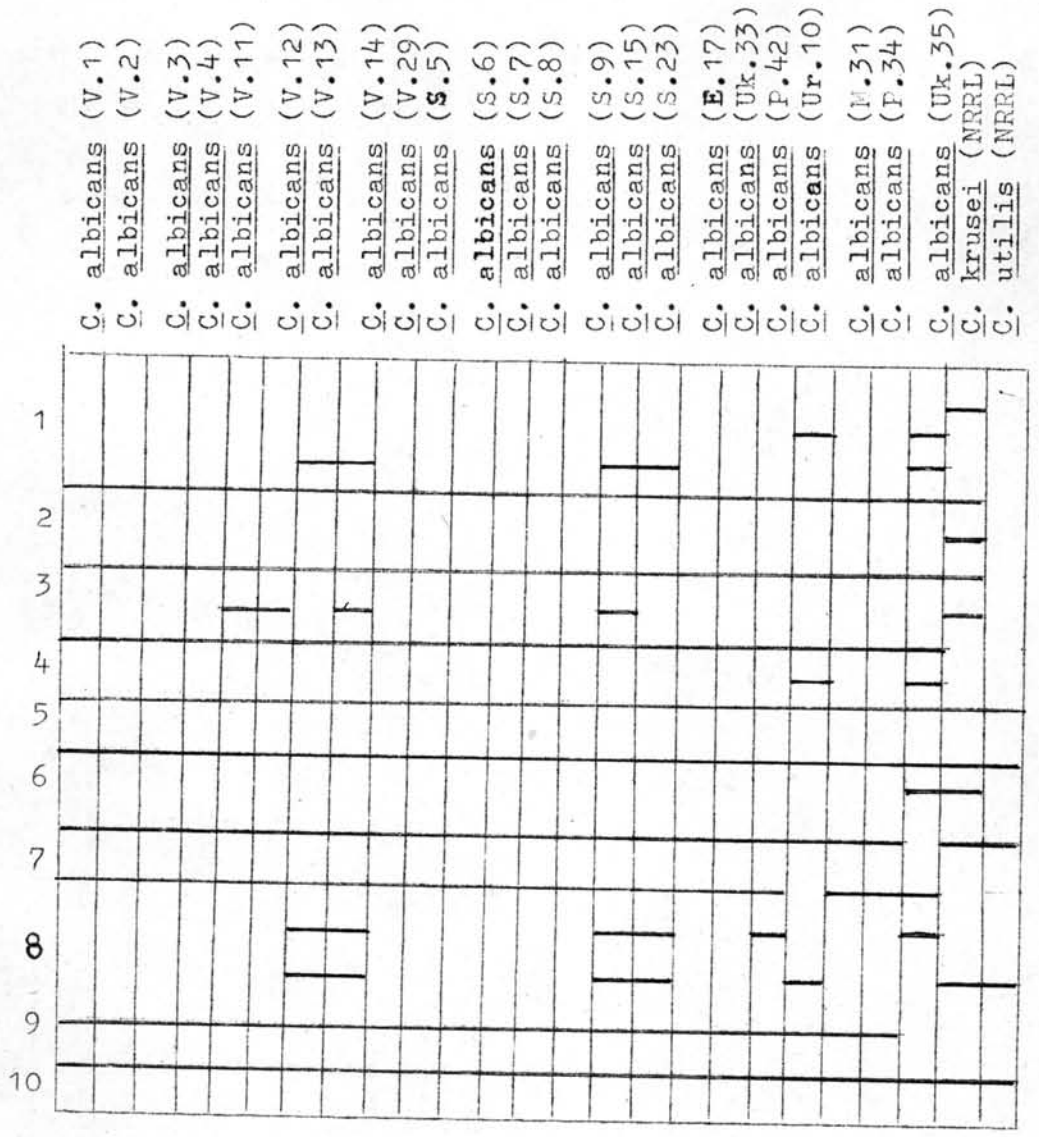

Figure 12 Electrophoretic pattern of soluble protein of twenty-three pathogens, Candida albicans, and two saprobes, arrangement in group based on lesions.

Abbreviation: V. = vagina; S. = sputum; E = ear;
M. = mouth; Uk. = Unknown location;
Ur. = Urine; P. = skin

showed the bands at the Rf 1.08, 7.47 and 8.12 which were considered matching with strains No. 6,7 of the sputum group and also strain unknown No. 2 which was subcultured from unknown location.

In twenty-three strains studied there were only two strains in various locations group, one isolated from urine and the other one from unknown location, had different protein patterns from the others. There were only eight common bands in urine isolate and the bands at the Rf 0.57, 4.08 were common with unknown strain No. 2. The difference between urine isolate and unknown strain No. 2 was that urine isolate possessed the band at the Rf 8.12 but the latter did not and had three more bands than urine isolate at Rf 1.08, 5.56 and 7.47.

The isolate of skin No. 2 had ten bands. The band at Rf 7.47 was common to the band of No. 8,9 of vaginal strain and strains No. 6,7 of sputum strain in figure 13.

Nine of matching bands were detected in the genus. There were four bands at the Rf 4.47, 5.24, 6.30 and 9.69 which considered to be matching among C. albicans, C. krusei, and C. utilis as shown in figure 13. C. krusei had two more bands at the Rf 1.65 and 2.71 which commoned with C. albicans.

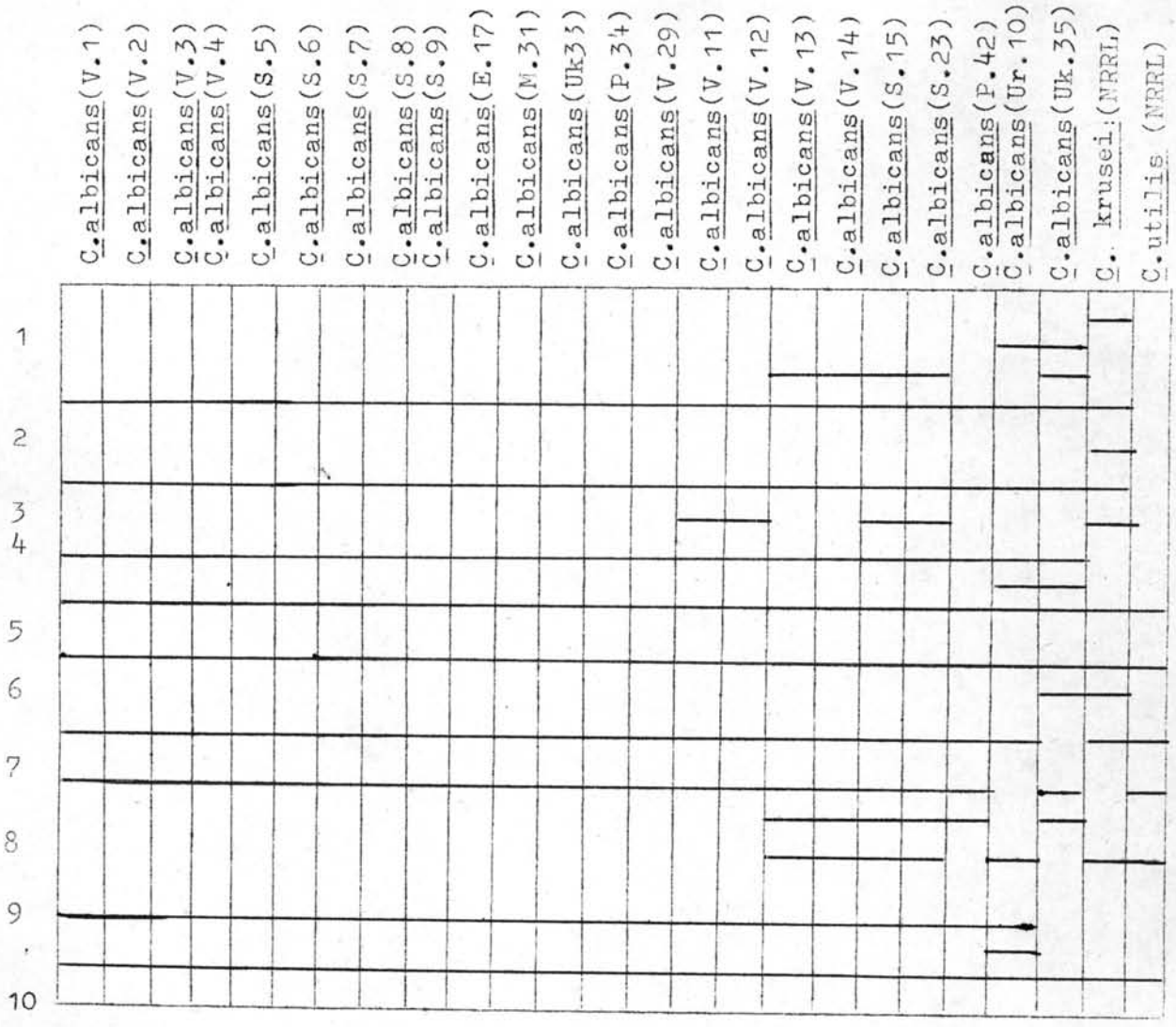

Figure 13 Pattern of gel column arrangement based on common protein bands of 23 strains of C.albicans and two saprobes.

Abbreviation: V.= vagina; S. = sputum; E. = ear; M. = mouth; Uk.= unknown; Ur. = urine; P. = skin.

The result of localized amylase activity by disc electrophoresis which followed Beneke's method (1975) was shown in figure 14. A colorless band was located in the blue gel electrophoretic column after staining with Gram's iodide which indicated that starch molecule was digested.

All twenty-three isolates of C. albicans,and two saprobes C. krusei and C. utilis showed amylase activity at the same Rf value. Only single clear band was detected at the Rf 0.3. Determination of amylase activity in the Candida species was carried out by using Sabouraud liquid medium and all isolates showed nothing difference in the number of bands and their activities.

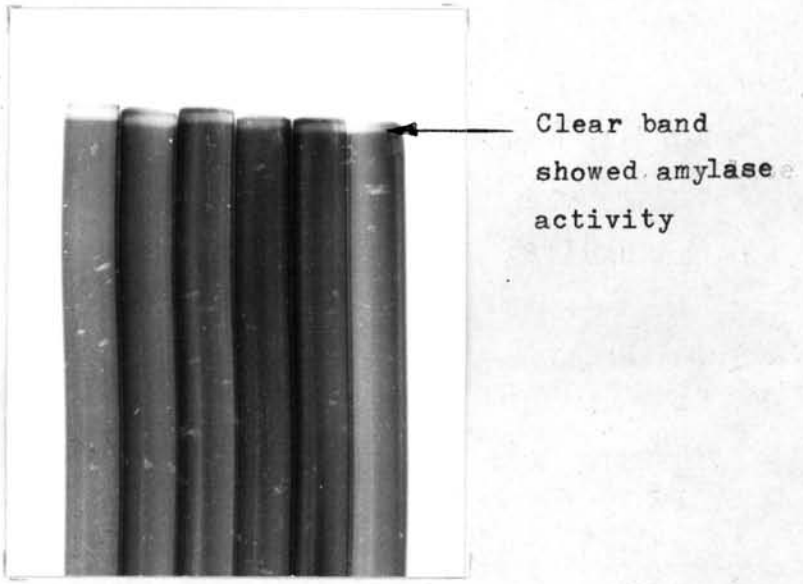

Figure 14 Photograph of gel column showed amylase activity when incubated with specific starch substrate.