CHAPTER V

RESULTS

1. Sample size characteristics

Three hundred thirty-six pigeon dropping samples were collected from 21/50 districts from Bangkok during June 2003 to April 2004. Most of the samples were found in temples (Wats) and in public parks. In Wats, the samples were scattered on the ground, wall and roof around shrine (figure 1, 2)

2. Isolation and identification of *Cryptococcus neoformans*

All the undiluted and diluted samples were plated on Sabouraud dextrose agar plus chloramphenicol (SC) and on differential medium, caffeic acid, as described in material and methods. *C. neoformans* were found from 10 out of 21 districts, not from 11 districts (figure 3, table 2). One-ten samples from 2-4 locations were these districts were taken (table 1). Only 1-2 colonies from samples from all districts except Samphantawong were shown. Among these ten districts, the isolated organisms from the samples vary from 6.25 to 81.25%. Samphantawong district was found to have *C. neoformans* 13 of 16 (81.25%) more than other districts. Min Buri and Thon Buri districts were found *C. neoformans* 3 of 16 (18.75%). Pasri Jaroen, Ladprow, Yannawa, and Bang Plad were equally found *C. neoformans* 2 of 16 (12.50%). Phayathai, Pranakorn, and Pomprab Satrupai were found *C. neoformans* 1 of 16 (6.25%).

Sampantawong district was the predominant district because *C. neoformans* was isolated with the highest percentage (81.25%), that was Wat Kusonsamakorn 4 of 4 (100%),Wat Bopitpimuk 6 of 8 (75%), and Wat Trimitt 3 of 4 (75%). *C. neoformans* was isolated in Wat Makok 1 of 3 (33.3%) in Phayathai. *C. neoformans* was isolated in Wat Khunjan 1 of 8 (12.5%) and Wat Yang Bangjak 1 of 5 (20%) in Pasri Jaroen.Wat Chanasongkram 1 of 4 (25%) in Pranakorn, Wat Bampen Nai 3 of 10 (30%) in Min Buri, Wat Sirikamonravat 2 of 9 (22.2%) in Ladpraw, Wat Dokmai 2 of 7 (28.5) in Yannawa, Wat Werurachin 3 of 6 (50%) in Thon Buri, and Wat

Paorohit 2 of 6 (33.3%) in Bang Plad were found *C. neoformans* in various percentage. Balee Obromtham school 1 of 4 (25%) was found *C. neoformans* in Pomprab Satrupai. The selected Public Park, road, university, and pet shop were not found *C. neoformans* in this study.

3. Variety test

Based on CGB medium, fifty two isolates were not able to utilize glycine as carbon source, resulting, no change of the bromthymol blue indicator, except one isolate (figure 4). This result showed that 52 isolates were *C. neoformans* var. *grubii/neoformans*, and one isolate was *C. neoformans* var. *gattii* (table 2). The result from CGB medium has a limitation to variety level, can not be separated *C. neoformans* var. *grubii* from *C. neoformans* var. *neoformans*, but the final results of variety were concluded from the molecular typing (in the lower detail).

4. Genetic diversity study

To demonstrate the diversity of these yeasts, RAPD using M13 as single primer was performed. The results showed that 50 isolates were *C. neoformans* var. *grubii* molecular type VNI which indicated serotype A, 2 isolates were *C. neoformans* var. *grubii* molecular type VNII which indicated serotype A, and 1 isolate was *C. neoformans* var. *gattii* (serotype B or C) (figure 12,table 2). Fifty two isolates were *C. neoformans* var. *grubii* and 1 isolate was *C. neoformans* var. *gattii* .VNI was the predominant molecular type, comprising 50 of 53 isolates (94.30%). VNII was recovered 2 of 53 isolates (3.77%). VNIII and VNIV were not detected. *C. neoformans* var. *gattii* was recovered only 1 of 53 isolate (1.88%) in Pomprab Satrupai.

The diversity among *C. neoformans* molecular type VNI was detected by the difference of the band numbers referred to the molecular type reference strains (figure 5, 6, 7). Type VNI showed the vary number of bands (6-12 bands) ranged from 1,800 to 350 bp. The major specified bands of VNI approximately were 1,780, 1,550, 1,380, 1,250, 1,000, 900, 800, 700, 600, 540 and 450. VNII (*C. neoformans* var. *grubii*, serotype A) was recovered 2 of 53 isolates (3.77%). VNII showed 6-10 bands ranged from 1,400 to 350 bp. The major specified bands of VNII approximately were

1,400, 1,250, 800, 700, 600, 540 and 450. VNIII and VNIV were not found in this study. *C. neoformans* var. *gattii* showed 10 bands ranged from 1,988-532 bp (table 3).

The genetic diversity of *C. neoformans* was classified by using number of bands in VNI and VNII. Subtype was divided into 7 subtypes, VNI.1, VNI.2, VNI.3, VNI.4, VNI.5, VNI.6 and VNI.7 in VNI and 2 subtypes, VNII.1, and VNII.2 in VNII. Subtype VNI.4 was the predominant subtype and subtype VNI.1, VNII.1, and VNII.2 were the minor subtype in this study. Table 4 came from the original data from table 3 and show schematic representation of the RAPD profile in figure 8, 9, 10, 11.

Table 1 Distribution of *Cryptococcus neoformans* in 21 districts in Bangkok

Districts	No. of positive* / No. of total samples (% positive samples)	Code of isolates
1. Phayathai	1/16 (1.25)	
1.1 Panita apartment	0/5 (0)	-
1.2 Wat Prayayoung	0/5 (0)	-
1.3 Wat Paiton	0/3 (0)	-
1.4 Wat Makok	1/3 (33.3)	PHA1
2. Pasri Jaroen	2/16 (12.5)	
2.1 Wat Nimmanoradee	0/3 (0)	-
2.2 Wat Khunjan	1/8 (12.5)	PAS1
2.3 Wat Yang Bangjak	1/5 (20)	PAS2
3. Pranakorn	1/16 (6.25)	
3.1 Thammasart University	0/5 (0)	-
3.2 Wat Bovonnivetviharn	0/3 (0)	-
3.3 Sanam Luang	0/4 (0)	-
3.4 Wat Chanasongkram	1/4 (25)	PRA1, PRA1.1
4. Min Buri	3/16 (18.75)	
4.1 Wat Bangpeng Tai	0/3 (0)	-
4.2 Wat Sansuk	0/3 (0)	-
4.3 Wat Bampen Nai	3/10 (30.0)	MIN1, MIN2, MIN3
5. Ladpraw	2/16 (12.5)	
5.1 Wat Ladpraw	0/7 (0)	-
5.2 Wat Sirikamonravat	2/9 (22.2)	LAD1, LAD1.1, LAD1.2, LAD1.3, LAD2.1, LAD2.2,
6. Yannawa	2/16 (12.5)	
6.1 Wat Thongbon	0/9 (0)	-
6.2 Wat Dokmai	2/7 (28.5)	YAN1, YAN2, YAN2.1

Table 1 Distribution of *Cryptococcus neoformans* in 21 districts in Bangkok (continued)

Districts	No. of positive* / No. of total samples (% positive samples)	Code of isolates
7. Pomprab Satrupai	1/16 (6.25)	
7.1 Wat Disanukaram	0/7 (0)	-
7.2 Wat Sakate	0/5 (0)	-
7.3 Balee Obromtham school	1/4 (25)	POM1
8. Sampantawong	13/16 (81.25)	
8.1 Wat Bopitpimuk	6/8 (75)	SAM1, SAM1.1, SAM2, SAM2.1, SAM3, SAM3.1, SAM4, SAM4.1, SAM4.2, SAM5, SAM6
8.2 Wat Trimitt	3/4 (75)	SAM7, SAM7.1, SAM7.2, SAM7.3, SAM7.4, SAM8, SAM8.1, SAM8.2, SAM8.3, SAM9
8.3 Wat Kusonsamakorn	4/4 (100)	SAM10, SAM10.1, SAM11, SAM11.1, SAM12, SAM13, SAM13.1, SAM13.2
9. Thon Buri	3/16 (18.75)	
9.1 Wat Werurachin	3/6 (50)	THO1, THO2, THO3, THO 3.1
9.2 Wat Doa Khanong	0/5 (0)	-
9.3 Wat Santithamaram	0/5 (0)	-
10. Bang Plad	2/16 (12.5)	
10.1 Wat Bavonmongkon	0/5 (0)	-
10.2 Wat Bang Yeekhan	0/5 (0)	-
10.3 Wat Paorohit	2/6 (33.3)	BAN1, BAN2
11. Taling Chan	0/16 (0)	
11.1 Wat Intraravat	0/5 (0)	-
11.2 Wat Keaw	0/4 (0)	-
11.3 Wat Paknam Phungnai	0/7 (0)	-

Table 1 Distribution of *Cryptococcus neoformans* in 21 districts in Bangkok (continued)

Districts	No. of positive* / No. of total samples (% positive samples)	Code of isolates
12. Bang Kapi	0/16 (0)	
12.1 Wat Sri Rungroeung	0/8 (0)	-
12.2 Wat Thepleela	0/8 (0)	-
13. Chatuchak	0/16 (0)	
13.1 Chatuchak park	0/4 (0)	-
13.2 Chankasem University	0/5 (0)	-
13.3 Rod Fai park	0/7 (0)	-
14. Patumwan	0/16 (0)	
14.1 Lumpinee park	0/6 (0)	-
14.2 Wat Hua Lampong	0/5 (0)	-
14.3 Srilom Road	0/5 (0)	-
15. Bang Bon	0/16 (0)	
15.1 Rungroeung Pet Shop	0/5 (0)	-
15.2 Wat Ninsukharam	0/5 (0)	-
15.3 Wat Bang Bon	0/6 (0)	-
16. Dusit	0/16 (0)	
16.1 Chitlada Palace Poad	0/5 (0)	-
16.2 Wat Benjamabopit	0/5 (0)	-
16.3 Wat Makutkasat	0/6 (0)	-
17. Bangkok Noi	0/16 (0)	
17.1 Wat Rakankositaram	0/5 (0)	-
17.2 Wat Plangvipadsana	0/5 (0)	-
17.3 Wat Nai Rong	0/6 (0)	-

Table 1 Distribution of *Cryptococcus neoformans* in 21 districts in Bangkok (continued)

Districts	No. of positive* / No. of total samples (% positive samples)	Code of isolates
18. Jom Thong	0/16 (0)	
18.1 Wat Rakankositaram	0/6 (0)	-
18.2 Wat Plangvipadsana	0/7 (0)	-
18.3 Wat Sai	0/3 (0)	-
19. Klong Sarn	0/16 (0)	
19.1 Wat Sawettachat	0/4 (0)	-
19.2 Wat Suwan	0/6 (0)	-
19.3 Wat Suttaram	0/6 (0)	-
20. Bang Khunthien	0/16 (0)	
20.1 Wat Khampang	0/7 (0)	-
20.2 Wat Hua Krabue	0/4 (0)	-
20.3 Wat Lauw	0/5 (0)	-
21. Bang Kae	0/16 (0)	
21.1 Wat Muang	0/4 (0)	-
21.2 Wat Sri Nuan	0/6 (0)	-
21.3 Wat Udomrangsri	0/6 (0)	-

* Number of the samples which *C. neoformans* was detected.

Table 2 Variety and Molecular type of *C. neoformans* from 21 Bangkok districts

District	No. of positive samples / No. of collected samples (% positive sample)	Variety* (No. of isolates)	Molecular type / No. of isolates
1. Phayathai	1/16 (6.25)	*grubii / neoformans* (1)	VNI (1)
2. Pasri Jaroen	2/16 (12.50)	*grubii / neoformans* (2)	VNI (2)
3. Pranakorn	1/16 (6.25)	*grubii / neoformans* (2)	VNI (2)
4. Min Buri	3/16 (18.75)	*grubii / neoformans* (3)	VNI (3)
5. Ladpraw	2/16 (12.50)	*grubii / neoformans* (6)	VNI (6)
6. Yannawa	2/16 (12.50)	*grubii / neoformans* (3)	VNI (3)
7. Pomprab Satrupai	1/16 (6.25)	*gatti* (1)	-
8. Sampantawong	13/16 (81.25)	*grubii / neoformans* (29)	VNI (28), VNII (1)
9. Thon Buri	3/16 (18.75)	*grubii / neoformans* (4)	VNI (3), VNII (1)
10. Bang Plad	2/16 (12.50)	*grubii / neoformans* (2)	VNI (2)
11. Taling Chan	0/16 (0)	-	-
12. Bang Kapi	0/16 (0)	-	-
13. Chatuchak	0/16 (0)	-	-
14. Patumwan	0/16 (0)	-	-

Table 2 Variety and Molecular type of *C. neoformans* from 21 Bangkok districts (continued)

District	No. of positive samples / No. of collected samples (% positive sample)	Variety* (No. of isolates)	Molecular type / No. of isolates
15. Bang Bon	0/16 (0)	-	-
16. Dusit	0/16 (0)	-	-
17. Bangkok Noi	0/16 (0)	-	-
18. Jom Thong	0/16 (0)	-	-
19. Klong Sarn	0/16 (0)	-	-
20. Bang Khunthien	0/16 (0)	-	-
21. Bang Kae	0/16 (0)	-	-
Total	30/336 (8.9)	*grubii / neoformans* (52), *gatti* (1)	VNI (50), VNII (2), *gatti* (1)

* Variety was identified by the growth on Canavanine glycine bromothymol blue medium.

Table 3 Serotype, molecular types and molecular sizes of major bands of each Cryptococcal isolates

Code	Variety (serotype)	Molecular types	Band No.	Molecular sizes of major bands (bp)
1. PHA1	*grubii* (A)	VNI	10	1790, 1544, 1393 1229, 986, 919, 788, 700, 577, 547
2. PAS1	*grubii* (A)	VNI	8	1804, 1520, 1395, 1231, 928, 800, 693, 575
3. PAS2	*grubii* (A)	VNI	9	1764, 1527, 1383, 1219, 1027, 900, 731, 648, 579
4. PRA1	*grubii* (A)	VNI	8	1816, 1578, 1404, 1239, 1060, 9[illegible], 805, 706
5. PRA1.1	*grubii* (A)	VNI	10	1803, 1579, 1414, 1247, 1007, 9[illegible]8, 794, 710, 616, 573
6. MIN1	*grubii* (A)	VNI	10	1731, 1349, 1190, 981, 893, 760, 664, 443, 318, 271
7. MIN2	*grubii* (A)	VNI	9	1812, 1562, 1401, 1236, 1017, 928, 812, 712, 585
8. MIN3	*grubii* (A)	VNI	11	1820, 1731, 1500, 1349, 1183, 987, 887, 765, 664, 557, 524
9. LAD1	*grubii* (A)	VNI	8	1803, 1567, 1403, 1238, 938, 800, 705, 590
10. LAD1.1	*grubii* (A)	VNI	10	1790, 1393, 1229, 1037, 906, 813, 759, 700, 568, 547

Table 3 Serotype, molecular types and molecular sizes of major bands of each Cryptococcal isolates (continued)

Code	Variety (serotype)	Molecular types	Band No.	Molecular sizes of major bands (bp)
11. LAD1.2	*grubii* (A)	VNI	6	1790,1500,1383, 1229, 1014, 925
12. LAD1.3	*grubii* (A)	VNI	10	1713, 1500, 1332, 1170, 1044, 887, 760,660, 553, 524
13. LAD2.1	*grubii* (A)	VNI	9	1807, 1570, 1408, 1233, 1030, 932, 802, 703, 569
14. LAD2.2	*grubii* (A)	VNI	9	1790, 1556, 1393, 1229, 1007, 932, 776, 705, 543
15. YAN1	*grubii* (A)	VNI	11	1803, 1584, 1420, 1248, 1148, 1042, 944, 805, 708, 475, 347
16. YAN2	*grubii* (A)	VNI	11	1800, 1583, 1419, 1248, 1170, 1036, 939, 806, 709, 476, 347
17. YAN2.1	*grubii* (A)	VNI	9	1764, 1559, 1383, 1230, 1009, 932, 823, 686, 584
18. POM1	*gattii*	-	10	1988, 1652, 1513, 1397, 1203, 1063, 1008, 772, 660, 532
19. SAM1	*grubii* (A)	VNI	8	1757, 1535, 1381, 1216, 912, 760, 678, 565
20. SAM1.1	*grubii* (A)	VNI	10	1798, 1592, 1410, 1248, 1105, 1043, 945, 791, 760, 568

Table 3 Serotype, molecular types and molecular sizes of major bands of each Cryptococcal isolates (continued)

Code	Variety (serotype)	Molecular types	Band No.	Molecular sizes of major bands (bp)
21. SAM2	*grubii* (A)	VNI	8	1780, 1486, 1383, 1219, 1027, 915, 800, 686
22. SAM2.1	*grubii* (A)	VNII	9	1390, 1226, 1111, 1029, 795, 689, 588, 542, 465
23. SAM3	*grubii* (A)	VNI	12	1820, 1688, 1481, 1307, 1154, 1098, 969, 869, 745, 650, 510, 437
24. SAM3.1	*grubii* (A)	VNI	9	1780, 1555, 1395, 1230, 932, 785, 693, 584, 545
25. SAM4	*grubii* (A)	VNI	8	1748, 1541, 1370, 1208, 1094, 1009, 915, 641
26. SAM4.1	*grubii* (A)	VNI	10	1747, 1521, 1329, 1024, 780, 715, 644, 596, 548, 526
27. SAM4.2	*grubii* (A)	VNI	9	1568, 1329, 1069, 867, 751, 636, 574, 515, 492
28. SAM5	*grubii* (A)	VNI	10	1560, 1210, 1055, 1008, 852, 740, 629, 572, 482, 424
29. SAM6	*grubii* (A)	VNI	7	1551, 1201, 1055, 740, 625, 572, 501
30. SAM7	*grubii* (A)	VNI	8	1790, 1544, 1393, 1229, 1022, 938, 700, 573

Table 3 Serotype, molecular types and molecular sizes of major bands of each Cryptococcal isolates (continued)

Code	Variety (serotype)	Molecular types	Band No.	Molecular sizes of major bands (bp)
31. SAM7.1	*grubii* (A)	VNI	9	1732, 1527, 1358, 1197, 1009, 915, 758, 667, 535
32. SAM7.2	*grubii* (A)	VNI	8	1774, 1581, 1409, 1248, 1037, 808, 592, 477
33. SAM7.3	*grubii* (A)	VNI	9	1811, 1561, 1400, 1236, 1017, 933, 801, 704, 593
34. SAM7.4	*grubii* (A)	VNI	9	1803, 1567, 1403, 1238, 1029, 938, 800, 705, 547
35. SAM8	*grubii* (A)	VNI	9	1764, 1522, 1373, 1211, 1007, 917, 776, 687, 531
36. SAM8.1	*grubii* (A)	VNI	7	1709, 1580, 1408, 1248, 1044, 803, 474
37. SAM8.2	*grubii* (A)	VNI	9	1804, 1398, 1232, 1018, 924, 804, 682, 591, 545
38. SAM8.3	*grubii* (A)	VNI	10	1773, 1571, 1383, 1227, 1021, 920, 783, 648, 453, 338
39. SAM9	*grubii* (A)	VNI	9	1780, 1569, 1395, 1241, 1036, 948, 792, 693, 559
40. SAM10	*grubii* (A)	VNI	11	1581, 1559, 1399, 1232, 1134, 1031, 936, 804, 702, 512, 468

Table 3 Serotype, molecular types and molecular sizes of major bands of each Cryptococcal isolates (continued)

Code	Variety (serotype)	Molecular types	Band No.	Molecular sizes of major bands (bp)
41. SAM10.1	*grubii* (A)	VNI	8	1777, 1533, 1383, 1220, 993, 925, 765, 687
42. SAM11	*grubii* (A)	VNI	9	1820, 1557, 1407, 1242, 1030, 937, 817, 701, 601
43. SAM11.1	*grubii* (A)	VNI	7	1799, 1516, 1391, 1228, 917, 803, 690
44. SAM12	*grubii* (A)	VNI	8	1809, 1560, 1399, 1235, 931, 804, 702, 587
45. SAM13	*grubii* (A)	VNI	9	1815, 1565, 1403, 1238, 1019, 927, 801, 704, 589
46. SAM13.1	*grubii* (A)	VNI	11	1699, 1463, 1324, 1168, 1104, 795, 881, 750, 655, 553, 517
47. SAM13.2	*grubii* (A)	VNI	8	1828, 1569, 1408, 1241, 1027, 882, 706, 569
48. THO1	*grubii* (A)	VNI	6	1796, 1583, 1408, 1286, 1000, 700
49. THO2	*grubii* (A)	VNII	6	1394, 1230, 1028, 924, 789, 703
50. THO3	*grubii* (A)	VNI	9	1788, 1539, 1395, 1230, 1024, 930, 708, 686, 574

Table 3 Serotype, molecular types and molecular sizes of major bands of each Cryptococcal isolates (continued)

Code	Variety (serotype)	Molecular types	Band No.	Molecular sizes of major bands (bp)
51. THO3.1	*grubii* (A)	VNI	11	1759, 1547, 1383, 1228, 1030, 929, 790, 695, 514, 462, 343
52. BAN1	*grubii* (A)	VNI	9	1780, 1583, 1395, 1275, 1046, 932, 785, 700, 589
53. BAN2	*grubii* (A)	VNI	8	1790, 1567, 1403, 1238, 1037, 945, 800, 705

Table 4 Diversity of pigeon droppings isolate based on the RAPD profile (classified by the number of bands and 100 bp differentiation)

Molecular types	**Subtype (No. of bands)**	**No. of group (No. of isolates)**
VNI	1 (12)	1(n=1)
VNI	2 (11)	5(n=6)
VNI	3 (10)	9(n=9)
VNI	4 (9)	10(n=17)
VNI	5 (8)	12(n=12)
VNI	6 (7)	3(n=3)
VNI	7 (6)	2(n=2)
VNII	1 (9)	1(n=1)
VNII	2 (6)	1(n=1)
var. *gattii*	1 (10)	1(n=1)

Figure 1 Specimens collection technique.

Figure 2 Pigeon droppings scattered on the side way around Wats.

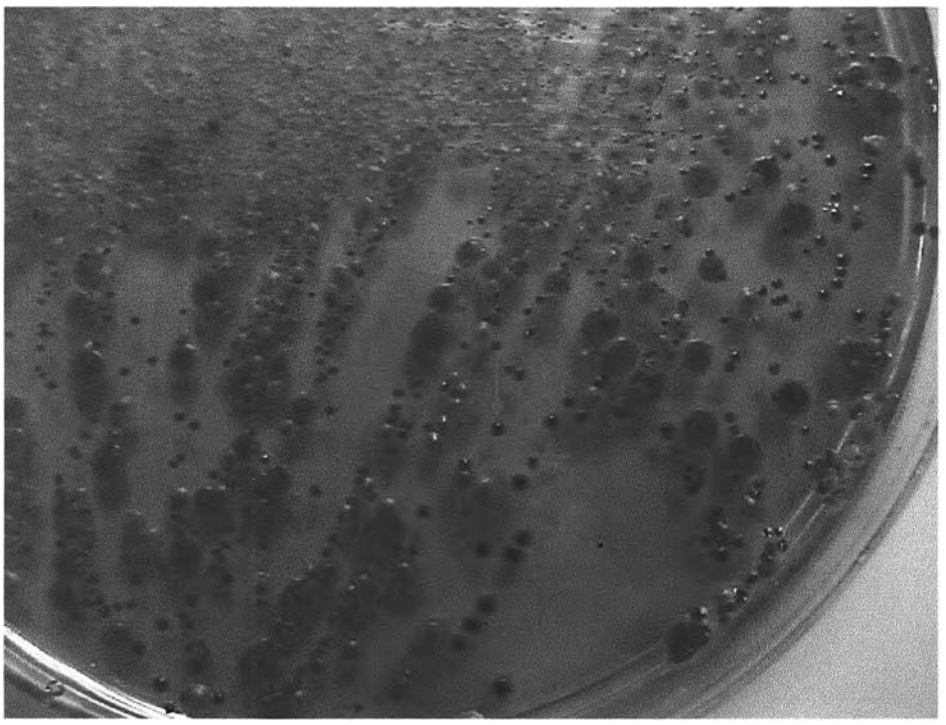

Figure 3 Dark brown colonies on the surface of caffeic acid agar at 7 days after inoculation.

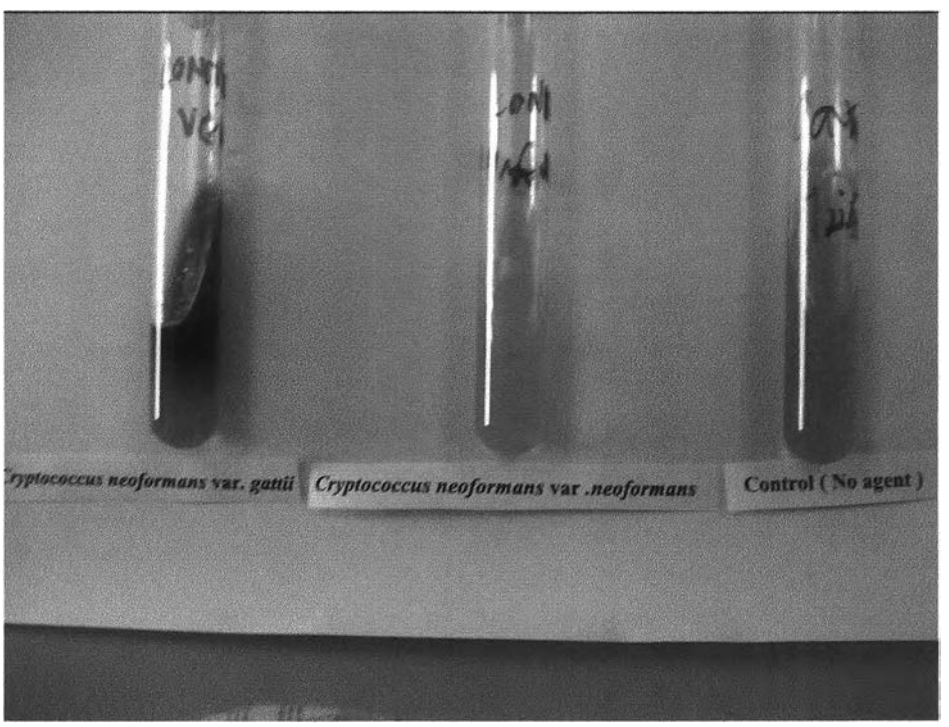

Figure 4 *C. neoformans* var. *gattii* changed the color of CGB agar to blue color whereas *C. neoformans* var. *grubii/neoformans* did not.

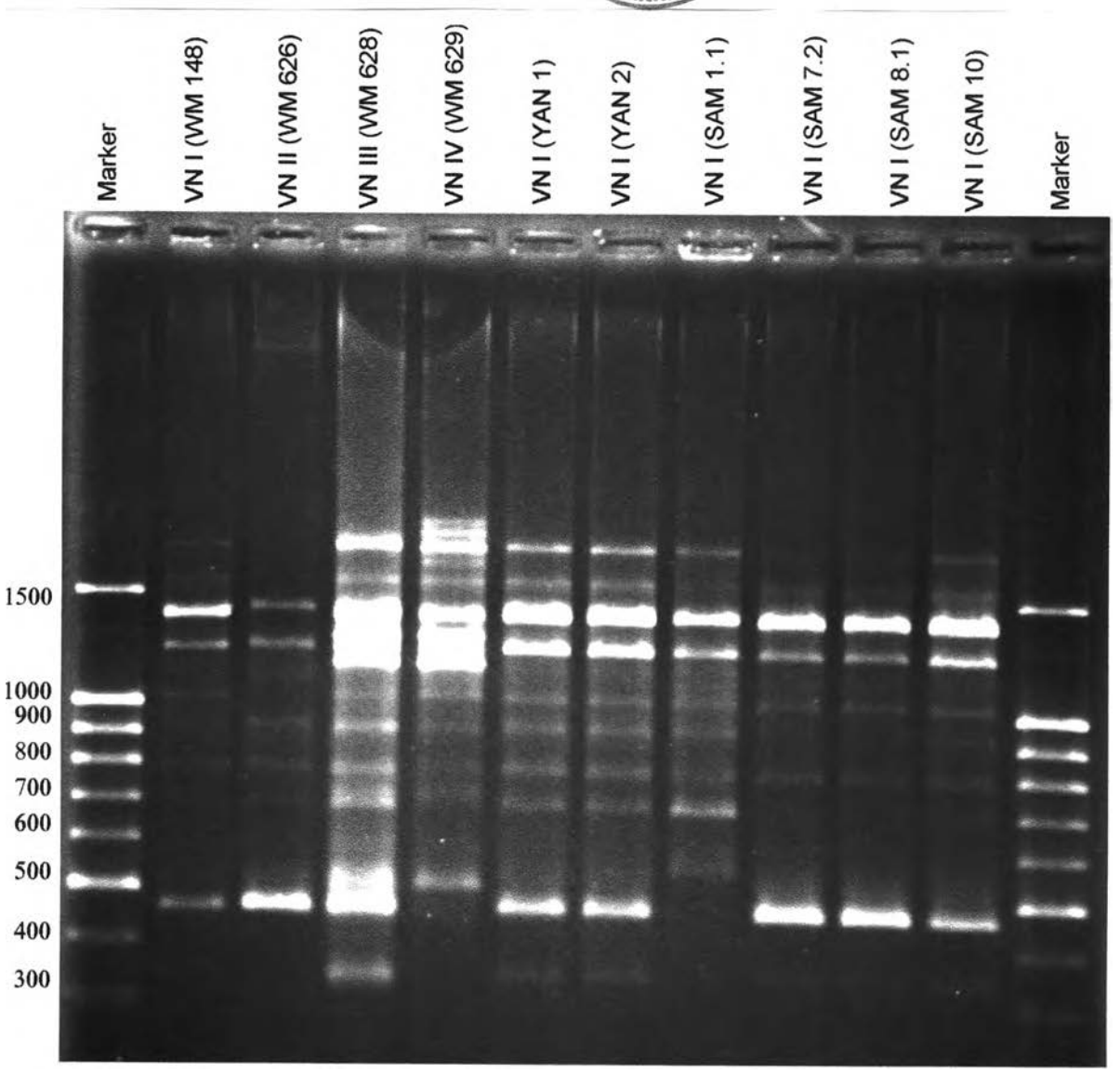

Figure 5 Random amplified polymorphic DNA generated with the M13 primer for identification of the molecular types. 100 bp - 1.5 kb marker was used. YAN1, and YAN2 obtained from Yannawa district. SAM 1.1, SAM7.2, SAM8.1, and SAM10 obtained from Samphantawong district. VNI (WM 148), VNII (WM 626), VNIII (WM 628), and VNIV (WM629) are standard molecular types.

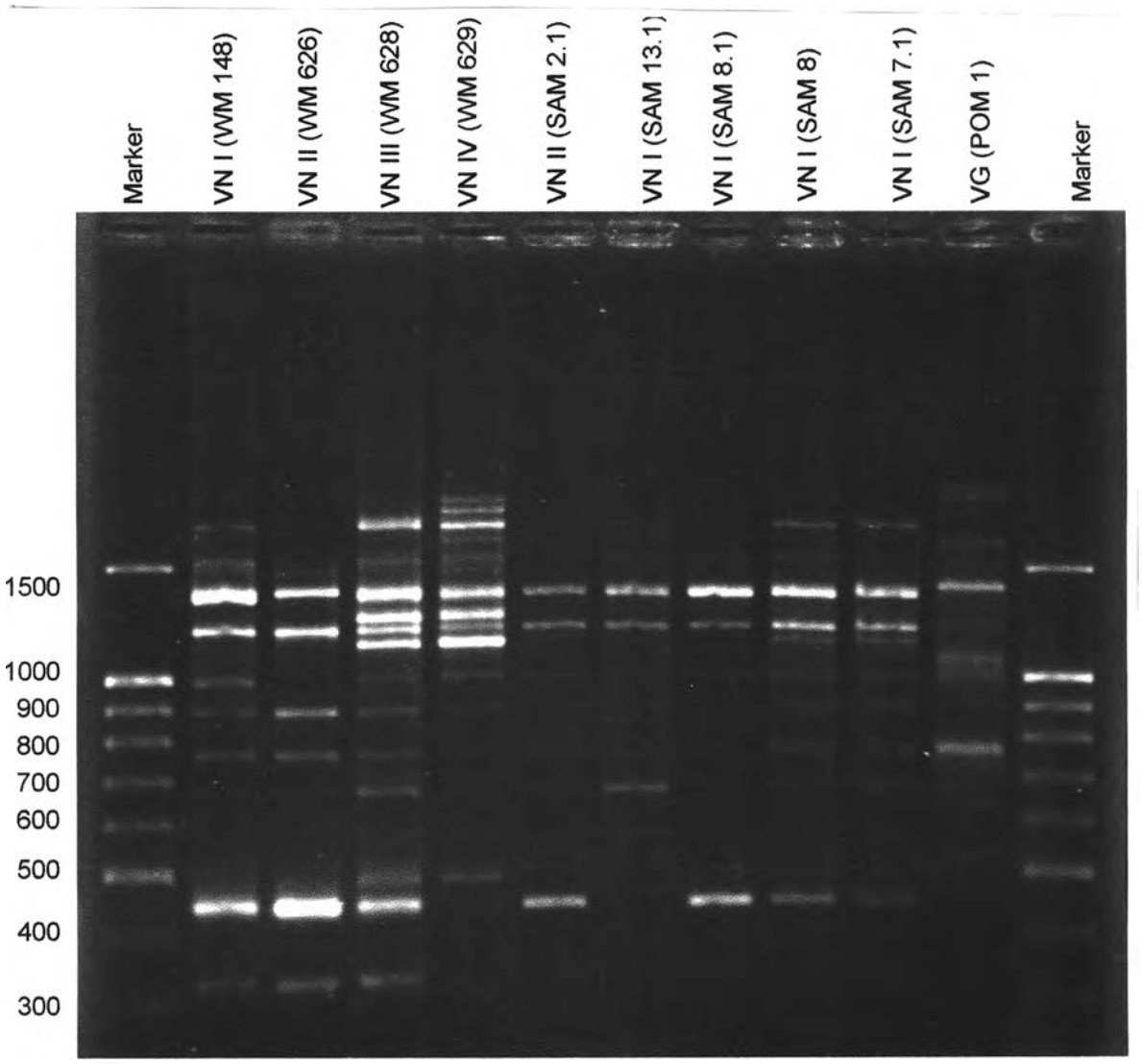

Figure 6 Random amplified polymorphic DNA generated with the M13 primer for identification of the molecular types. 100 bp - 1.5 kb marker was used. SAM 2.1, SAM13.1, SAM8.1, SAM8, and SAM7.1 obtained from Samphantawong district. POM1 obtained from Pomprab Satrupai. VNI (WM 148), VNII (WM 626), VNIII (WM 628), and VNIV (WM629) are standard molecular types.

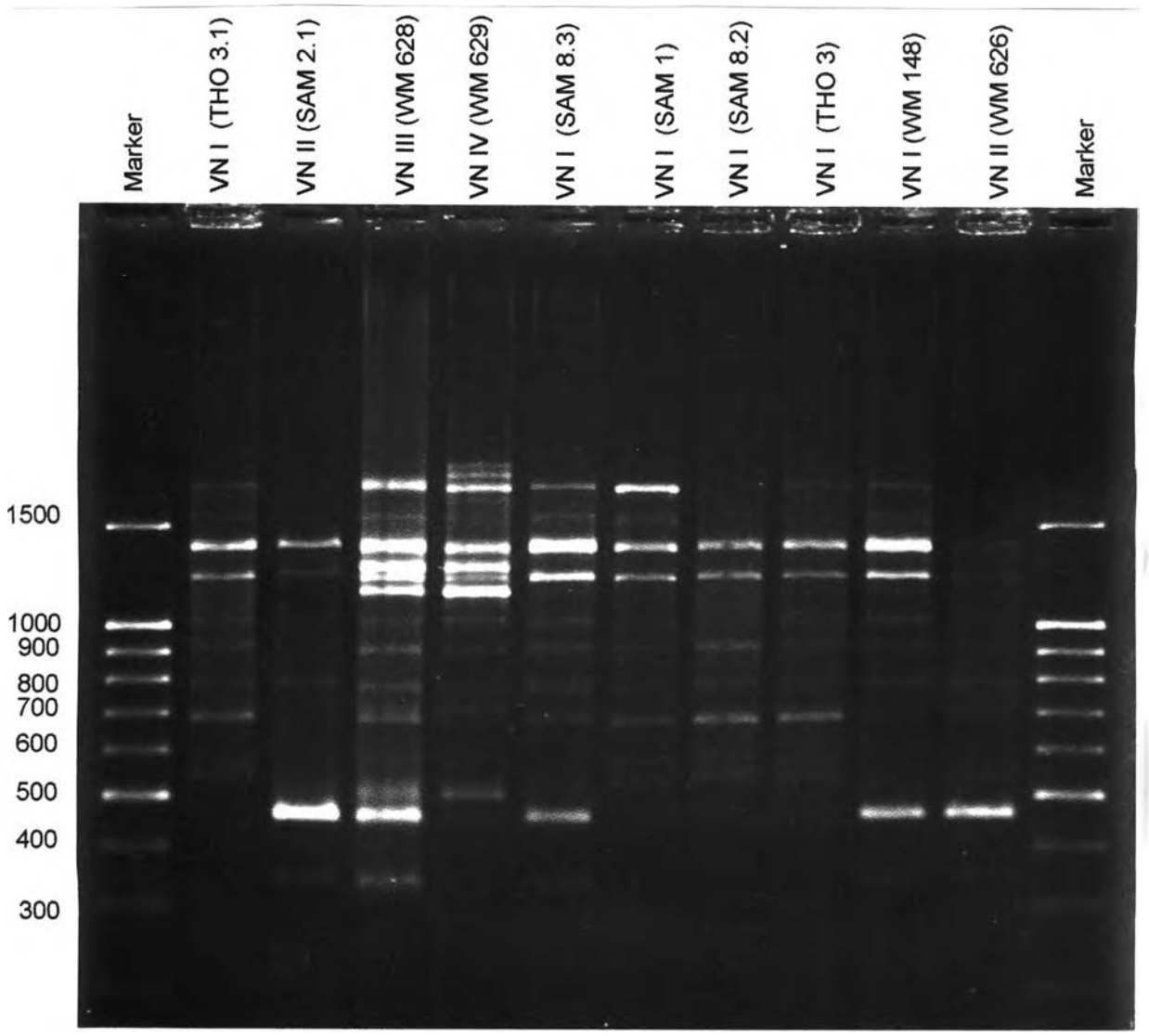

Figure 7 Random amplified polymorphic DNA generated with the M13 primer for identification of the molecular types. 100 bp - 1.5 kb marker was used. SAM2.1, SAM8.3, SAM1 and SAM8.2 obtained from Samphantawong district. THO3, and THO3.1 obtained from Thon Buri district. VNI (WM 148), VNII (WM 626), VNIII (WM 628), and VNIV (WM629) are standard molecular types.

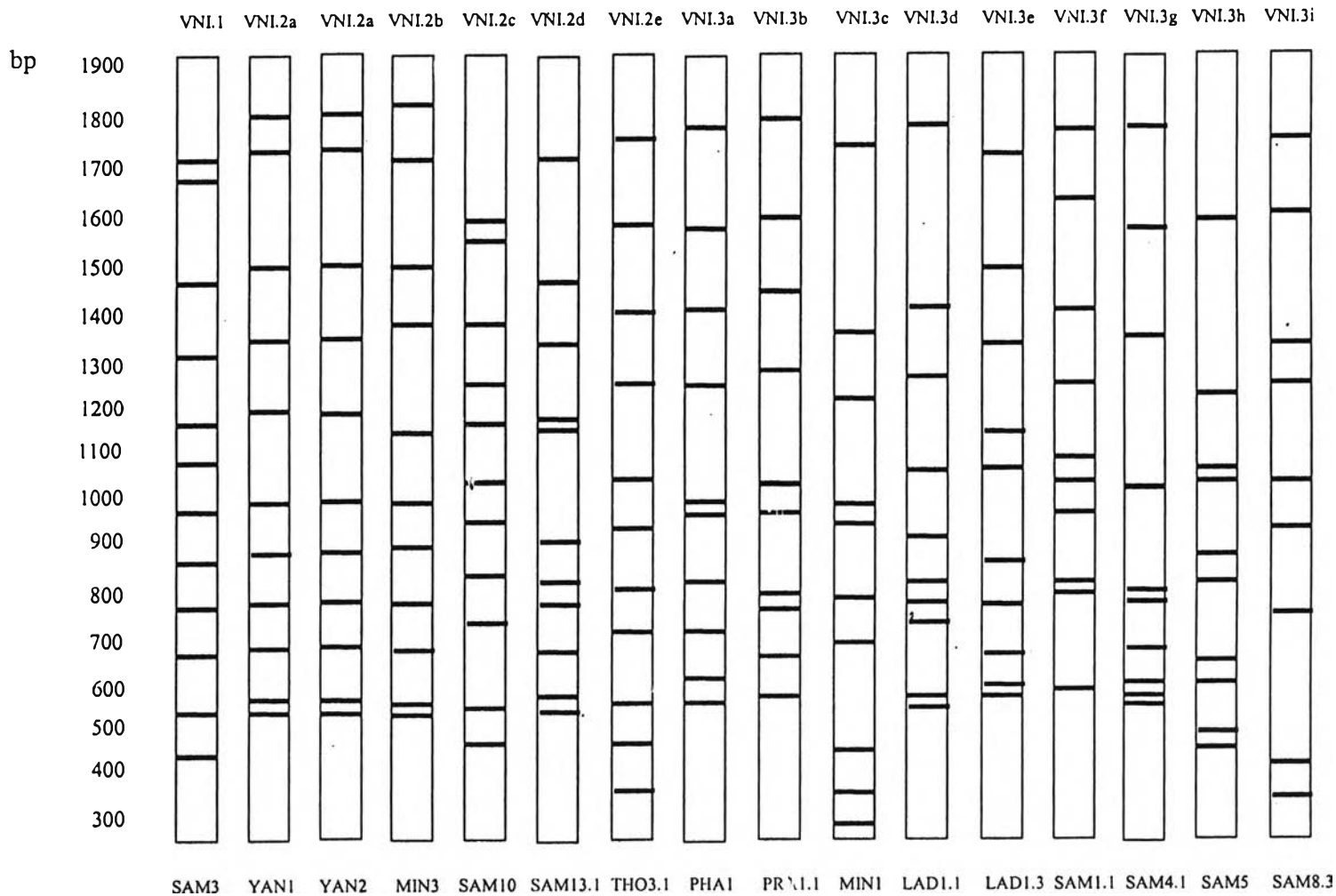

Figure 8 Schematic representation of the RAPD profile. The number on the left represented molecular sizes. The letter and number on the top represented molecular types, subtypes and groups. VNI mean *C. neoformans* var. *grubii*, molecular type VNI. The number after VNI showed subtypes and the small letter showed groups. The letter and numbe: on the bottom showed the locations.

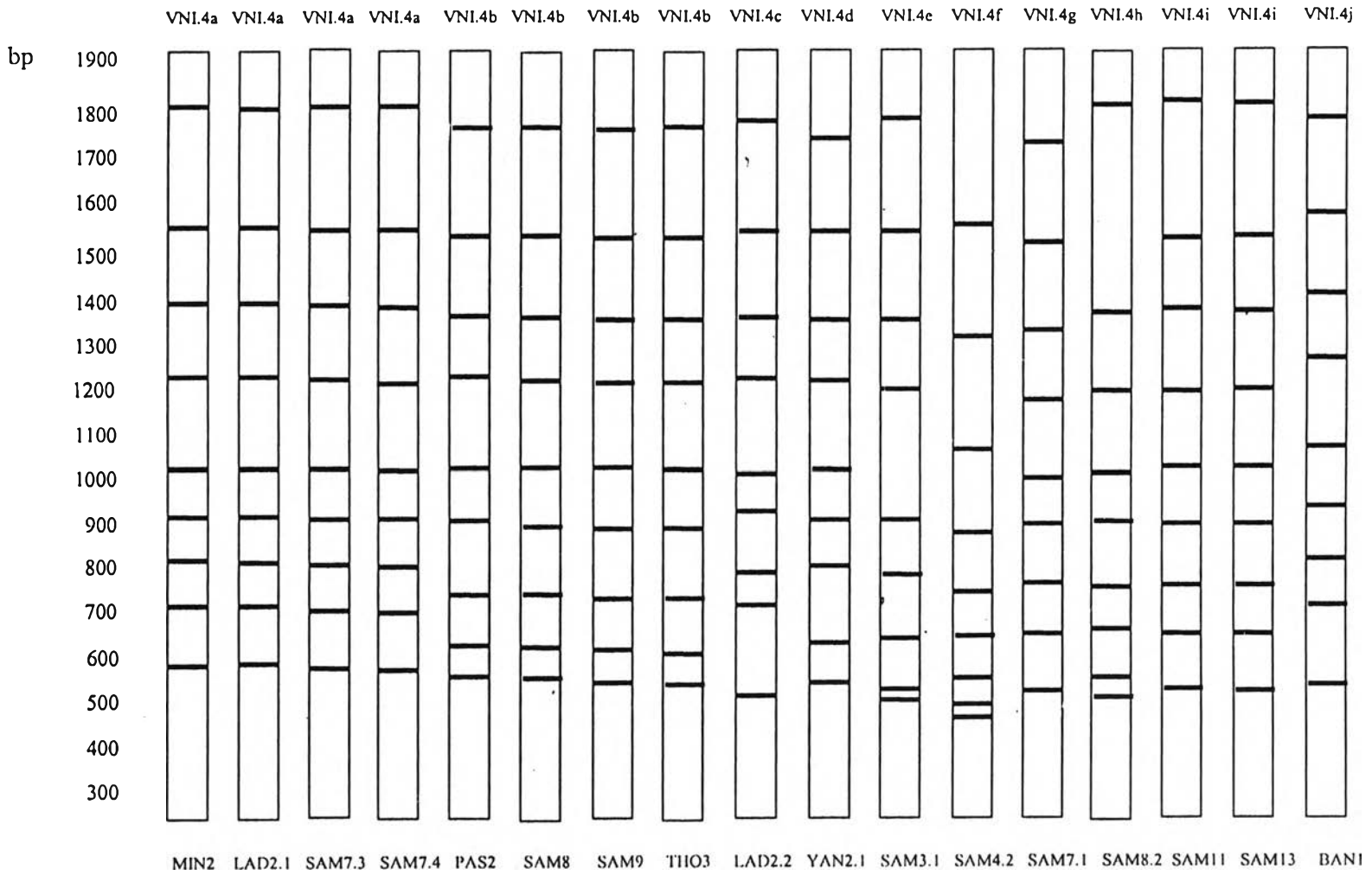

Figure 9 Schematic representation of the RAPD profile. The number on the left represented molecular sizes. The letter and number on the top represented molecular types, subtypes and groups. VNI mean *C. neoformans* var. *grubii*, molecular type VNI. The number after VNI showed subtypes and the small letter showed groups. The letter and number on the bottom showed the locations.

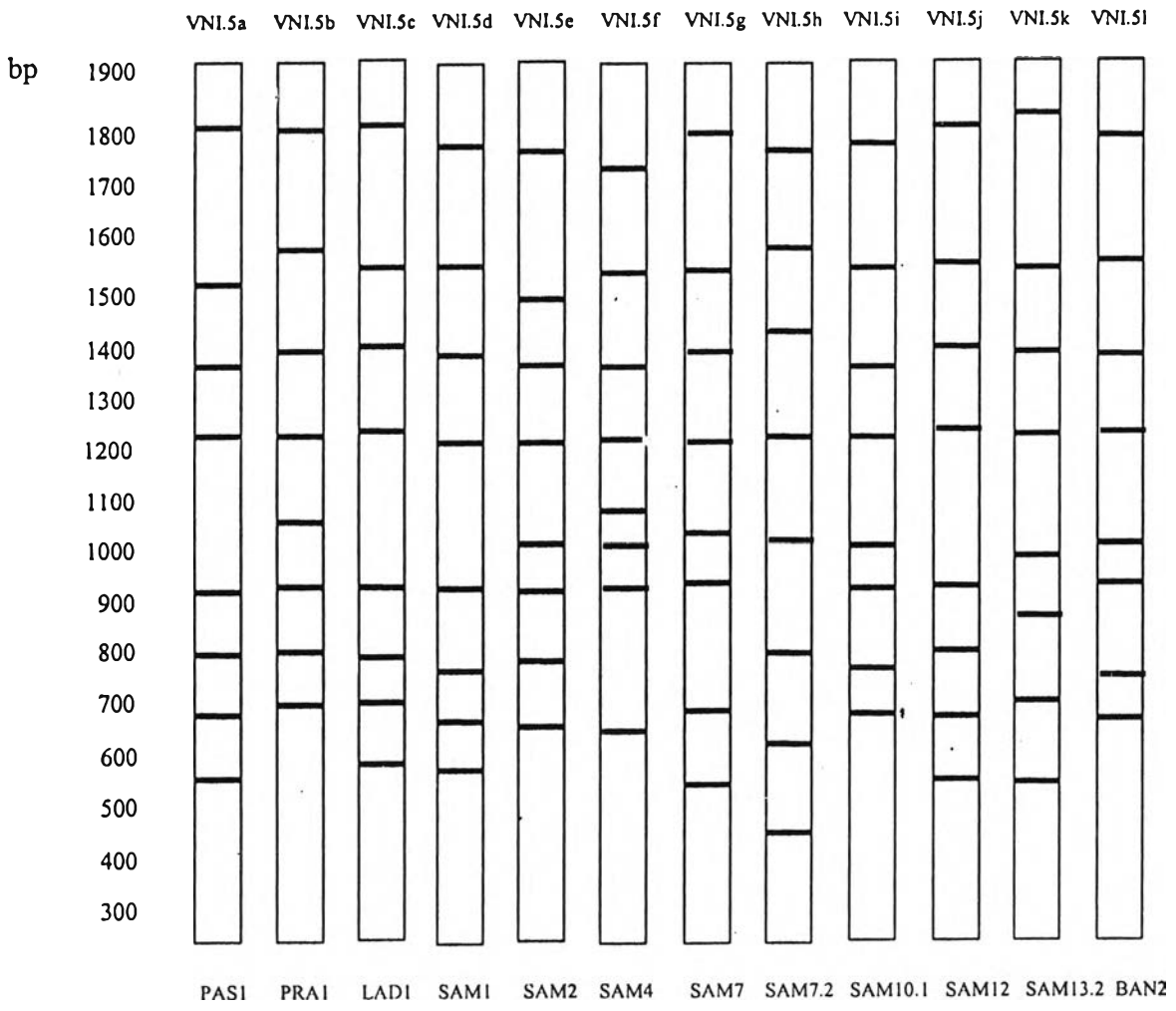

Figure 10 Schematic representation of the RAPD profile. The number on the left represented molecular sizes. The letter and number on the top represented molecular types, subtypes and groups. VNI mean *C. neoformans* var. *grubii*, molecular type VNI. The number after VNI showed subtypes and the small letter showed groups. The letter and number on the bottom showed the locations.

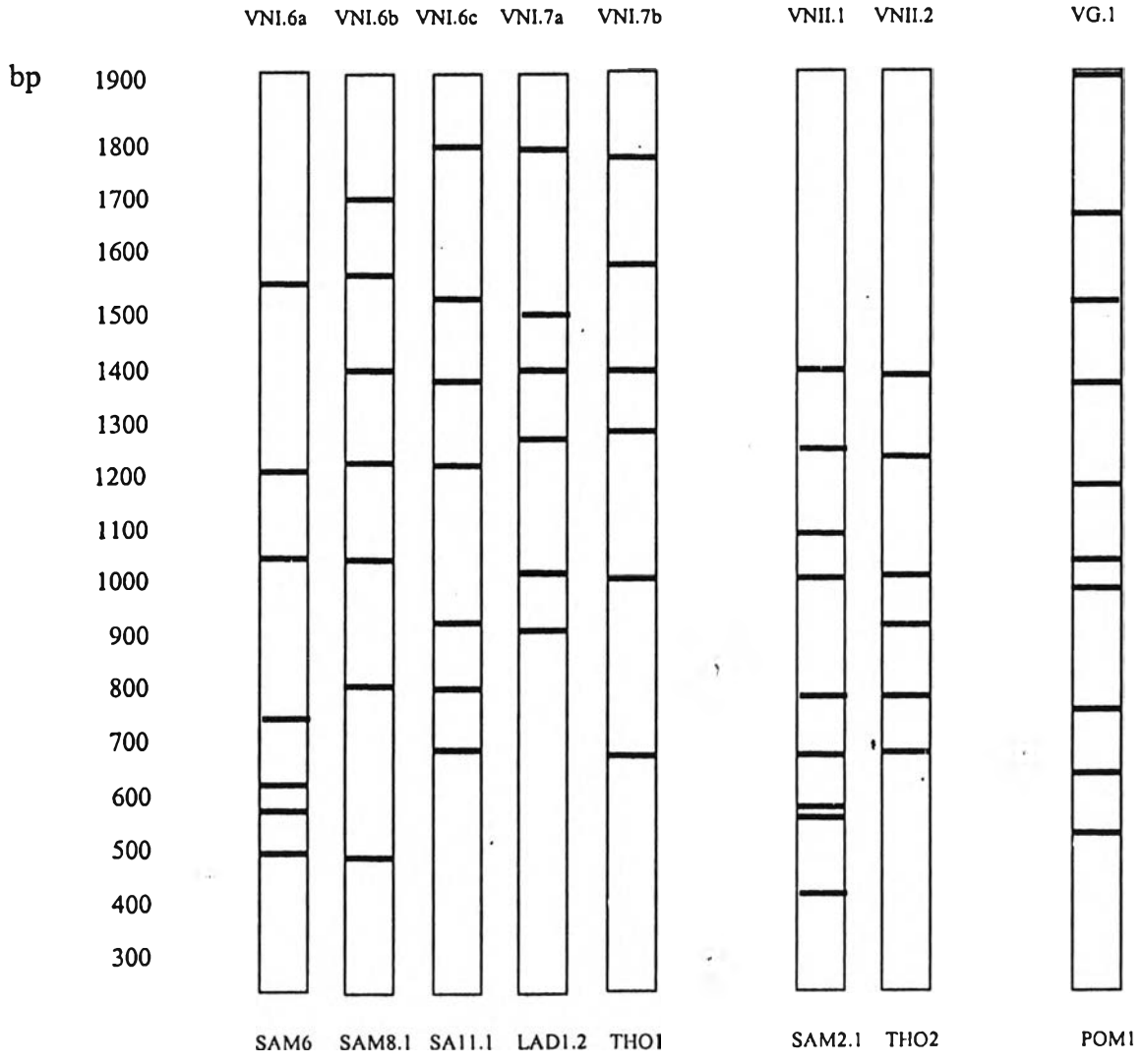

Figure 11 Schematic representation of the RAPD profile. The number on the left represented molecular sizes. The letter and number on the top represented molecular types, subtypes and groups. VNI and VNII mean *C. neoformans* var. *grubii*, molecular type VNI and VNII. VG mean *C. neoformans* var. *gattii*. The number after VNI, VNII, and VG showed subtypes and the small letter showed groups. The letter and number on the bottom showed the locations.

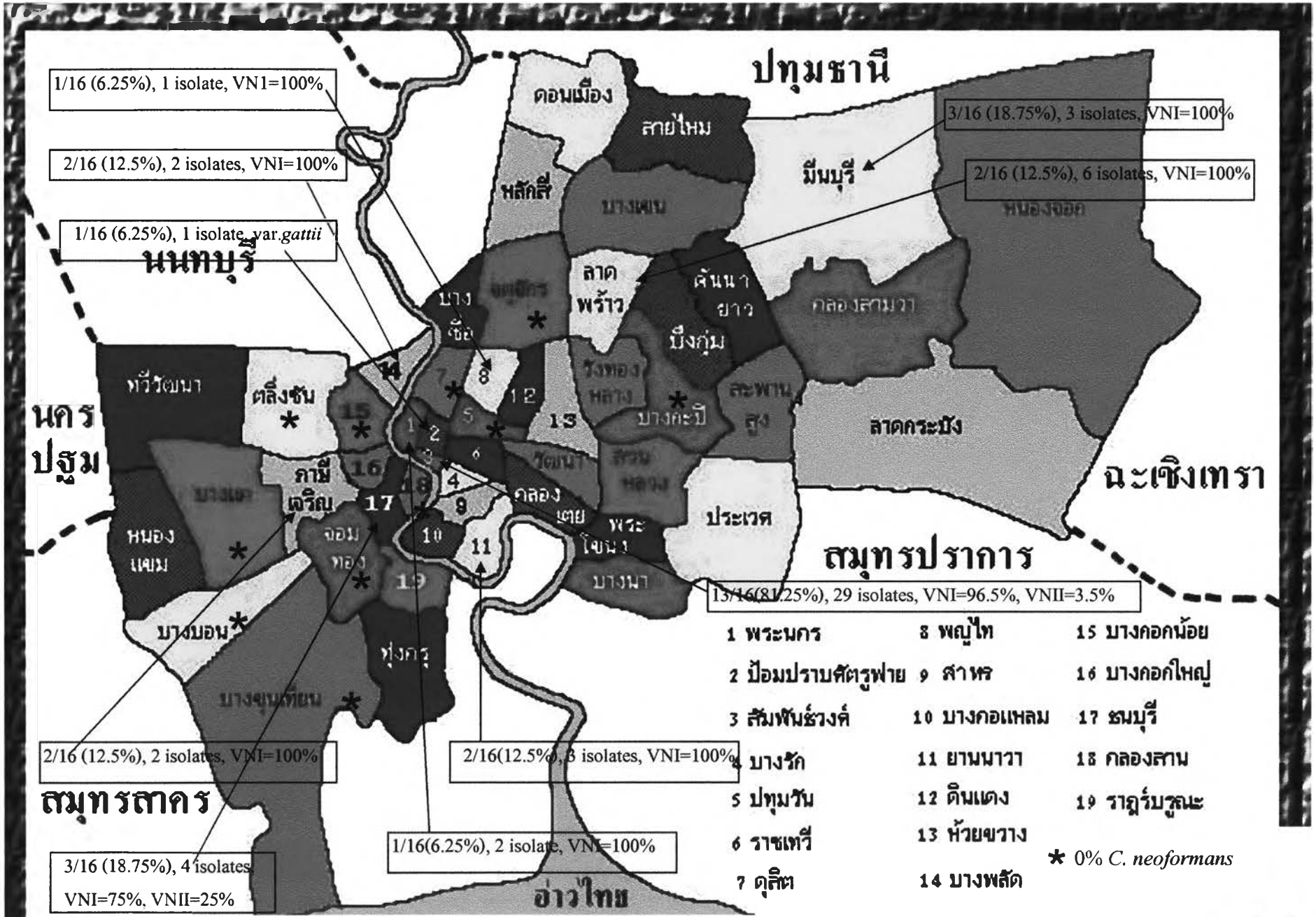

Figure 12 Geographic distribution of the molecular type in Bangkok