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Comparision of Polypeptide Patterns by 2-D PAGE in *Fusarium* Species

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이차 전기영동법을 이용한 *Fusarium*속의 다당류 비교

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Abstract: *F. napiforme*, *F. beomiforme* and *F. nygamai* could not be classified in any of the existing sections of the genus *Fusarium*. To discuss of the exact taxonomic relationships among these species, the cellular polypeptide patterns were compared by using 2-D PAGE. Polypeptide pattern of *F. beomiforme* was different from those of other two species and was more similar to *F. oxysporum* in section *Elegans*. *F. nygamai* and *F. napiforme* might be another same section which would lie between section Liseola and section Elegans. The results were consistent with the comparison of isoenzyme patterns in these species.

KEYWORDS: *F. napiforme*, *F. beomiforme*, *F. nygamai*, *F. oxysporum*, 2-D PAGE

The genus *Fusarium* are worldwide distribution and important pathogenic fungus in a variety of plant, animal, and human. *F. beomiforme* (Nelson *et al.*, 1987), *F. nygamai* (Burgess & Trimboli, 1986) and *F. napiforme* (Marasas *et al.*, 1987) are distinguished from species in section *Liseola* by the formation of chlamydospores and from species in section *Elegans* by mode of formation of microconidia. However, it is not a reliable criterion for separating species in section *Liseola* and *Elegans* and these three species because there is considerable overlap between species (Nelson *et al.*, 1990).

In order to discuss the exact taxonomic relationships, it would be necessary to understand the difference among the isoenzymes and proteins in these species. In a previous paper (Min and Kweon, 1994), isoenzyme patterns of these species were reported. In this study, the cellular protein were compared of these species using two-dimensional polyacrylamide gel electrophoresis (2-D PAGE).

For almost twenty years, 2-D PAGE has been represented a powerful method for resolving cellular extracts of protein (Norbeck and Blomberg, 1995). Its high resolution and sensitivity allow the detection of extremely small variation in charge and molecular weight. Protein similarities and differences have been used for taxonomical characterization of fungi, bacteria, and algae (Kawchuk *et al.*, 1987).

Material and Methods

F. beomiforme Nelson *et al.* 9758, *F. napiforme* Marasas *et al.* 6129, *F. nygamai* Burgess and Trimboli 5668, *F. oxysporum* Schlecht 7500, *F. moniliforme* Scheldon 7150, *F. subglutinans* Nelson 1082 and *F. graminearum* were received from Dr. L.W. Burgess in Australia. Cultures were maintained on PDA (Difco) at 28°C. Protein was extracted using a modified procedure of van Eatten *et al.* (1979) and Kawchuk *et al.*

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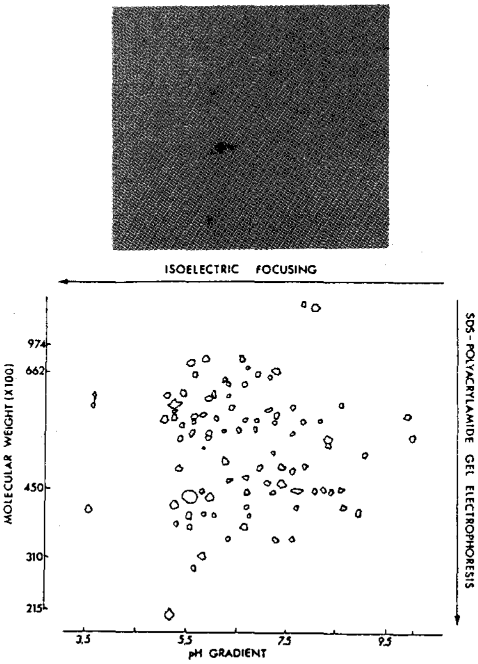

Fig. 1. *Fusarium oxysporum*

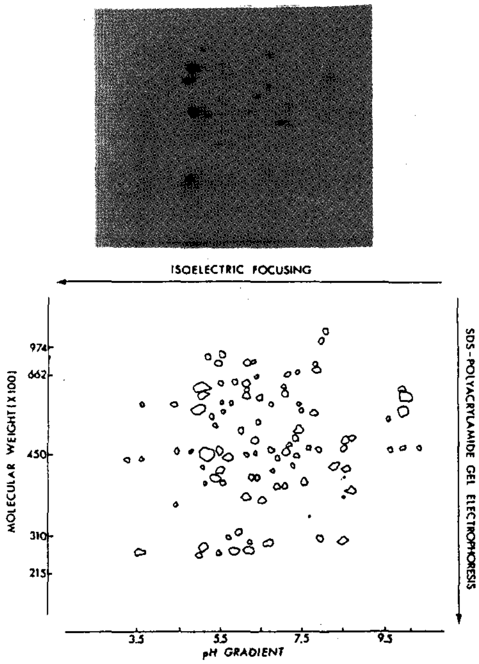

Fig. 2. *Fusarium graminearum*

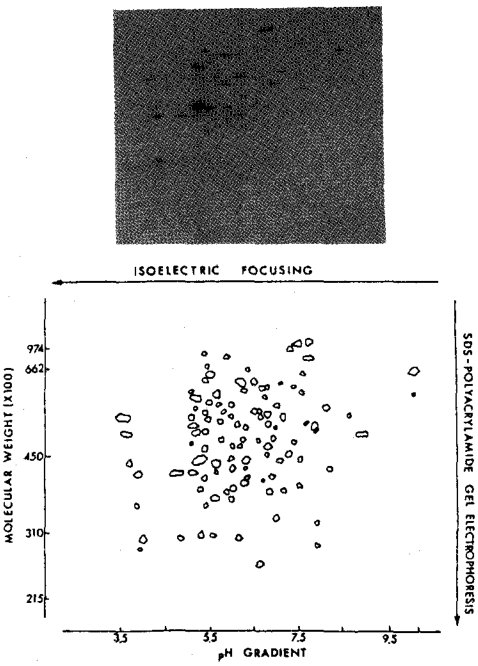

Fig. 3. *Fusarium subglutinans*

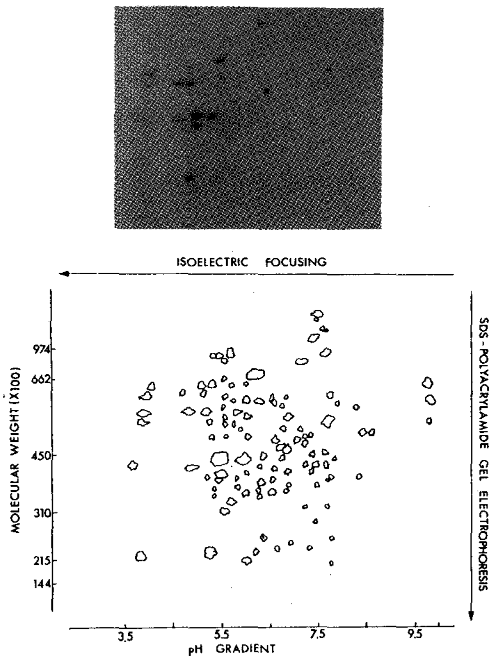

Fig. 4. *Fusarium moniliforme*

Fig. 1～7. Two-dimentional isoelectric focusing-polyacrylamide gel electrophoresis of polypeptide.
Upper: 2-D gel stained with Coomassie Blue
Lower: Diagramatic representation of polypeptide scanned by Image-Analyzer Computer

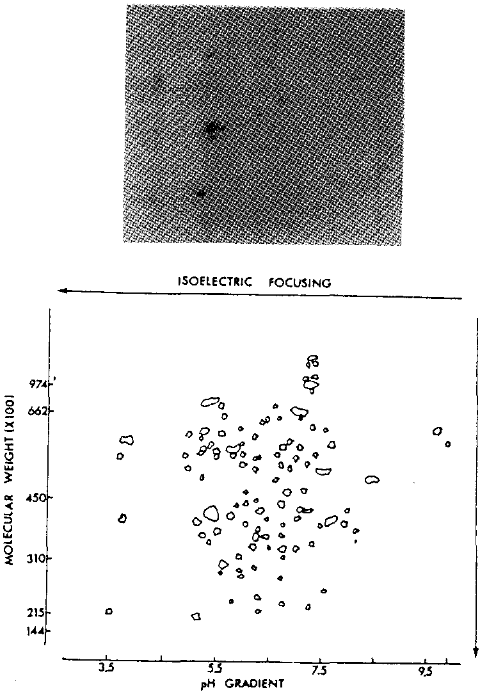

Fig. 5. *Fusarium nygamai*

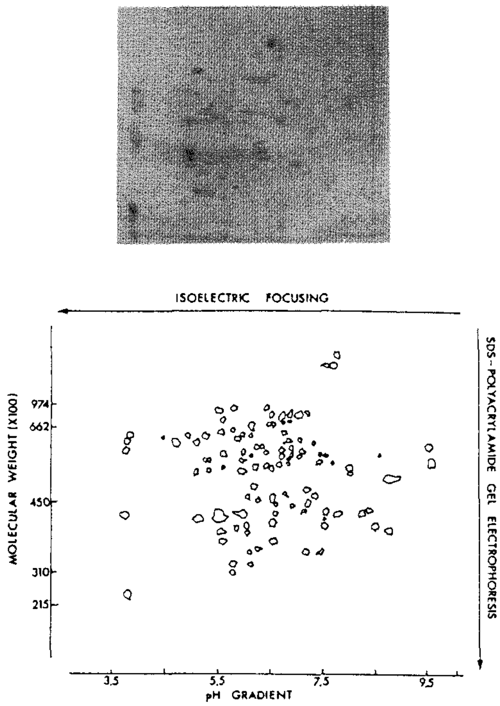

Fig. 6. *Fusarium beomiforme*

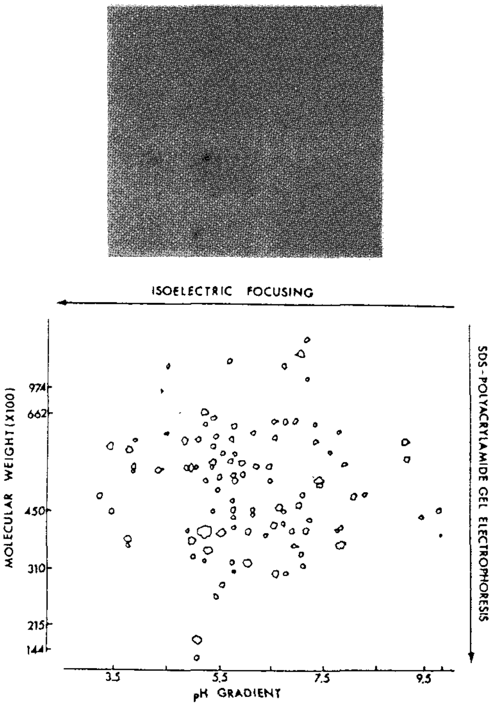

Fig. 7. *Fusarium napiforme*

(1987). 2-D PAGE was run a Hoefer system using a modified procedure of O'Farrell (1975) and Howes *et al.* (1982) with ali chemicals supplied by Sigma products.

As standard marker, phosphorylase b (M.W. 97,400), serum albumin (M.W. 66,200), o-valbumin (M.W. 45,000), carbonic anhydrase (M.W. 31,000), trypsin inhibitor (M.W. 21, 500), lysozyme (M.W. 14,400) were used. Gel staining was accomplished by gently shaking the gels overnight in a solution containing 0. 1% coomassie brilliant blue R-250, 25% isopropyl alcohol, 10% acetic acid. The gel was destained in a solution containing methyl alcohol : acetic acid : D.W. (5 : 1 : 4), and then in a solution containing methyl alcohol : acetic acid : D.W. (7 : 5 : 88). Destained gels were photographed and analyzed with CREAM Image Analysis System. Sample preparation and electrophoretic separation were repeated to acquire accurate results. In order

Table 1. Summary of protein matching among the seven species of *Fusarium* using 2-D protein profile (Fig. 1~7)

Spot number	Coordinates in master profile		Density in master profile	Presence in *Fusarium* spp.						
	X	Y		oxy	beo	nap	gra	sub	nyg	mon
1	20.5	28.4	63.94		+	+	+	+	+	
2	21.3	85.7	35.04		+	+	+	+	+	
3	21.4	56.8	75.38	+	+	+	+	+	+	+
4	22.1	61.5	34.11				+	+	+	
5	24.7	72.4	41.48					+	+	+
6	26.3	25.0	31.09		+		+	+	+	+
7	26.3	75.5	86.71			+		+		+
8	27.1	81.8	108.15	+	+	+	+		+	+
9	28.4	84.9	59.83	+	+	+		+		+
10	33.0	84.6	15.55		+	+	+		+	
11	37.3	82.4	93.77		+	+	+	+	+	
12	38.5	45.3	31.91						+	
13	39.1	64.6	36.77				+		+	
14	39.3	82.8	43.30		+			+		+
15	41.7	56.2	94.16		+	+				+
16	41.7	76.0	128.26			+				+
17	45.3	79.4	142.67		+	+	+		+	
18	45.8	85.4	66.55	+						
19	46.4	27.6	74.27	+			+	+	+	
20	46.6	82.8	55.49			+		+	+	
21	47.4	76.0	124.36	+	+	+		+	+	
22	47.7	88.0	140.83		+	+	+	+		
23	48.2	52.6	64.34			+	+	+	+	+
24	48.4	58.9	36.00		+	+	+	+	+	
25	48.7	25.5	135.14	+		+	+	+	+	+
26	49.0	72.9	47.70	+		+		+	+	+
27	49.5	67.2	50.34	+				+	+	+
28	49.7	85.9	75.05	+	+	+	+	+	+	+
29	50.0	45.8	39.04					+		+
30	50.0	96.4	47.62				+		+	+
31	50.3	48.4	47.09	+					+	+
32	51.3	51.0	82.30	+	+	+	+		+	+
33	51.3	56.3	34.79			+	+	+	+	
34	51.8	59.6	112.02	+	+	+	+	+	+	+
35	52.1	95.8	45.02		+			+		+
36	52.4	94.3	53.80		+		+	+	+	
37	52.7	51.1	42.97		+			+	+	
38	53.2	86.2	33.81		+			+	+	
39	53.4	53.6	112.48	+	+	+	+	+		+
40	53.4	87.5	38.54	+	+	+	+	+	+	+
41	53.6	78.1	39.69	+	+	+	+	+	+	+
42	53.8	91.1	19.97	+	+			+	+	
43	53.9	71.4	33.85			+				+
44	53.9	73.4	34.45	+	+	+	+	+	+	+
45	53.9	94.3	33.38	+						+
46	54.2	28.1	36.88	+	+	+	+	+	+	

Table 1. Contined

Spot number	Coordinates in master profile		Density in master profile	Presence in *Fusarium* spp.						
	X	Y		oxy	beo	nap	gra	sub	nyg	mon
47	54.2	66.7	29.36			+	+			+
48	54.4	40.6	41.02	+	+	+		+	+	+
49	54.4	81.8	38.30	+	+	+	+	+	+	+
50	55.2	57.8	39.53	+	+	+	+	+	+	
51	55.5	99.2	67.77		+	+	+	+	+	
52	56.0	96.4	58.66		+	+	+	+		+
53	56.2	44.3	62.35		+	+		+	+	+
54	56.5	84.9	34.15	+	+	+	+	+	+	+
55	56.8	70.8	29.16			+		+		+
56	57.0	80.2	25.79	+				+		+
57	57.1	95.4	39.10	+	+				+	
58	57.3	33.9	34.79	+		+	+		+	
59	57.3	36.5	30.35	+	+			+		
60	57.3	87.3	19.71	+	+					
61	57.8	52.1	33.00	+						+
62	57.8	75.5	45.45	+	+	+	+	+	+	+
63	58.3	49.0	31.36	+		+				+
64	59.1	69.3	26.64	+						+
65	60.2	89.6	42.27	+		+	+		+	
66	60.3	72.1	50.12	+	+					
67	60.7	23.4	48.02				+	+	+	+
68	60.8	85.7	19.77		+					
69	60.9	59.6	111.18	+	+	+	+	+	+	+
70	61.1	79.7	21.22		+				+	
71	61.2	35.9	39.92	+	+	+	+	+	+	
72	61.2	85.4	26.46	+	+	+	+	+	+	+
73	61.5	46.9	41.88			+		+	+	+
74	61.5	53.6	35.74	+	+			+	+	+
75	61.7	80.2	46.00	+		+		+		+
76	62.0	67.2	42.84	+	+	+	+	+	+	+
77	62.0	74.5	68.75	+	+	+	+	+	+	+
78	63.0	88.5	105.86		+	+	+	+		+
79	63.2	43.5	23.68		+				+	
80	63.8	85.1	19.23		+				+	
81	64.1	96.4	48.53				+			
82	64.8	26.6	43.00				+	+	+	+
83	65.4	32.3	27.43	+		+	+		+	
84	65.4	47.9	41.07			+	+	+		+
85	65.6	45.8	39.39	+				+		+
86	65.7	81.3	17.79		+					
87	65.9	79.7	72.03	+	+	+	+	+	+	+
88	66.1	56.8	40.54		+	+	+	+	+	+
89	66.4	51.0	63.02		+			+	+	+
90	66.9	68.8	55.15	+	+	+	+	+	+	
91	67.2	60.4	60.42	+					+	+
92	67.4	31.2	61.22				+		+	+
93	67.4	64.1	35.52	+		+	+		+	

Table 1. Contined

Spot number	Coordinates in master profile		Density in master profile	Presence in *Fusarium* spp.						
	X	Y		oxy	beo	nap	gra	sub	nyg	mon
94	67.6	81.3	16.24		+				+	
95	67.7	91.7	34.90	+			+	+	+	
96	68.5	75.0	35.67	+	+	+	+	+	+	
97	68.9	94.8	22.53		+					
98	69.2	74.3	28.38		+				+	
99	70.1	47.4	68.23	+			+		+	+
100	70.3	81.2	36.29	+	+	+	+	+	+	+
101	70.6	52.1	37.89	+					+	+
102	70.8	73.2	21.31		+					
103	70.8	92.7	21.73		+					
104	71.4	55.7	37.87	+	+	+	+	+	+	+
105	71.4	66.1	68.36		+			+		+
106	71.4	79.7	43.70	+	+	+	+	+		+
107	71.6	47.6	61.75		+				+	
108	71.9	27.6	32.54					+		+
109	72.4	63.0	46.13	+	+	+	+	+	+	+
110	73.4	59.9	31.65	+	+		+	+	+	+
111	73.7	78.6	46.44	+	+			+		+
112	74.0	70.3	32.68		+	+			+	+
113	74.0	91.6	25.96		+				+	
114	75.1	90.0	17.62		+					
115	75.3	47.9	45.07			+		+	+	+
116	75.3	62.5	59.30	+	+	+	+	+	+	+
117	75.3	81.2	35.24	+	+			+	+	+
118	75.7	83.0	19.44		+					
119	75.8	54.7	57.94				+	+	+	+
120	75.8	74.5	57.89	+	+	+		+	+	+
121	76.3	50.0	35.68		+		+		+	+
122	76.6	29.7	35.64				+		+	+
123	77.5	93.2	36.50		+					
124	77.8	81.3	19.40			+				
125	78.1	88.5	31.85	+	+	+	+	+	+	
126	78.1	88.5	31.85	+	+	+	+	+	+	
127	78.9	65.6	58.80	+	+	+	+	+		+
128	78.9	94.3	52.04	+	+	+	+	+	+	+
129	79.9	69.8	29.18	+	+		+		+	+
130	80.8	92.7	65.72			+				
131	81.1	83.5	16.90			+				
132	81.5	64.6	51.95	+	+		+	+	+	+
133	81.5	67.2	40.83				+		+	+
134	81.6	79.7	19.40			+				
135	81.8	42.2	26.77	+				+		+
136	82.0	57.3	45.06	+	+	+	+	+	+	+
137	82.6	93.2	43.23		+	+	+	+	+	
138	82.8	81.8	30.52			+	+	+		
139	82.8	27.6	31.21					+	+	+
140	83.1	72.7	125.30				+	+	+	

Table 1. Contined

Spot number	Coordinates in master profile		Density in master profile	Presence in *Fusarium* spp.						
	X	Y		oxy	beo	nap	gra	sub	nyg	mon
141	83.3	102.1	47.85					+	+	+
142	83.6	67.7	42.56	+	+	+	+	+		+
143	83.8	60.8	50.69			+				
144	83.9	54.7	39.29	+	+	+	+	+	+	+
145	83.9	79.7	40.70	+	+	+	+	+		
146	84.1	60.9	52.62	+					+	+
147	84.9	37.0	29.96							+
148	85.4	49.5	39.44	+	+	+			+	+
149	85.4	57.3	38.14			+			+	+
150	86.2	109.4	51.48							+
151	86.5	110.7	120.31	+	+			+		+
152	87.5	104.7	38.14				+		+	+
153	87.8	60.9	38.54	+	+	+				+
154	88.3	78.1	99.40	+			+	+	+	
155	88.3	95.8	39.11		+		+	+		+
156	88.5	104.2	35.58					+		+
157	89.1	42.2	28.54							+
158	89.1	57.3	49.17							+
159	89.3	51.6	34.43							+
160	90.1	65.6	53.28	+	+	+	+	+		
161	90.1	73.4	109.22		+	+		+	+	+
162	90.6	22.4	34.91					+		+
163	90.6	31.2	47.00				+		+	+
164	90.6	52.1	35.71		+					+
165	91.1	82.8	32.37		+	+		+	+	+
166	91.4	58.9	37.30	+	+				+	+
167	92.2	107.8	38.47	+	+	+	+		+	
168	92.7	78.4	27.70	+	+			+	+	
169	95.3	59.1	116.53	+	+	+	+	+	+	
170	98.4	32.3	29.65	+		+	+		+	
171	98.4	48.4	36.86			+	+	+		
172	98.7	65.6	61.00	+		+	+		+	
173	99.0	68.5	80.91			+	+		+	
174	99.2	58.3	96.42		+	+	+	+	+	
175	99.2	77.6	32.96		+	+		+		+
176	100.5	52.6	42.82	+	+	+	+	+	+	+
177	101.6	68.7	59.31				+		+	+
178	104.7	68.7	52.50	+	+	+		+		+
179	113.8	76.0	31.70				+		+	
180	114.8	65.1	37.20				+	+	+	
181	118.8	86.2	41.69		+	+	+	+	+	
182	124.2	80.7	39.50			+	+	+	+	+
183	124.2	85.9	87.43	+	+	+	+	+	+	+
184	124.7	72.4	35.63	+	+		+			+

oxy: *F. oxysporum*, beo: *F. beomiforme*, nap: *F. napiforme*, nyg: *F. nygamai*, sub: *F. subglutinans*, mon: *F. moniliforme*, gra: *F. graminearum*

Table 2. Polypeptide similarity for seven *Fusarium* species by 2-D electrophoresis

	oxy	beo	nap	nyg	sub	mon
beo	63.37					
nap	58.16	57.14				
nyg	54.90	55.96	66.98			
sub	57.84	57.80	54.72	59.09		
mon	61.54	55.86	56.48	63.39	70.54	
gra	53.40	52.68	54.27	54.11	53.14	51.19

oxy: *F. oxysporum*, beo: *F. beomiforme*, nap: *F. napiforme*, nyg: *F. nygamai*, sub: *F. subglutinans*, mon: *F. moniliforme*, gra: *F. graminearum*

to compare the similarity among the species, the patterns of polypeptides were analiyed with CREAM 2-D analytical software, version 4.1. The similarity values were caculated using the methods of Aquardro *et al.* (1981).

Results and Discussion

After the two-dimensional polypeptide profile of *Fusarium* seven species were traced (Fig. 1~7), numbers of spot detected on picture were compared and counted. The summary of the two-dimensional polypeptide pattern using computer analyzer (CREAM Image Analyzer) was given in Table 1. Several faint polypeptides were not always visible on the gel and were omitted from the comparisons.

The study was focused on determining differences and similarities of polypeptide pattern among the seven species. In order to determine the similarity of polypeptide among species in *Fusarium*, the difference between each two species was compared and matching protein percentage was calculated (Table 2). The polypeptide patterns between *F. moniliforme* and *F. subglutinans* in section *Liseola* were the highest similarity (70.54%). Among *F. nygamai*, *F. napiforme* and *F. beomiforme* which were known to have characters of both section *Liseola* and section *Elegans*, polypeptide of *F. beomiforme* was more similar to *F. oxysporum* which belonged to section *Elegans* (63.37%). The similarity of *F. napiforme* and *F. beomiforme* (57.14%) was lower values that was similar to those of *F. nygamai* and *F. beomiforme* (55.96%). The similarity of *F. graminearum* and other all remaining species was lower. This result coincided with that section *Discolor* including *F. graminearum* has been known to be more distant phylogenetic relationship from section *Liseola* and section *Elegans*.

The results of this experiment were similar with that of Ellis (1988) in which the comparison of genetic relatedness using DNA complementarity between *Fusarium* section was demonstrated. The genetic relatedness of *F. subglutinans* and *F. moniliforme* was shown the highest similarity (48~52%). The genetic relatedness of *F. moniliforme* and *F. oxysporum* was 42%. This indicated that these two species, *F. moniliforme* and *F. oxysporum*, were belonged to different section but were much more close phylogenic relationship. Those of *F. moniliforme* and *F. graminearum* was 12% which meant that these two species showed much more distant phylogenemetic relationship with other species.

F. beomiforme, F. nygamai and *F. napiforme* were distinguished from species in section *Liseola* by the formation of chlamydospores. They were differed from *F. oxysporum* primarily by the nature and the mode of formation of microconidia. (Nelson *et al.*, 1987). *F. napiforme* resembled *F. nygamai* in morphology but the absence of polyphialides and the presence of napiforme to lemon-shaped microconidia differenciated *F. napiforme* from *F. nygamai* (Marasas *et al.*, 1987). According to the comparative data of some morphological and physiological characters to temperature and osmotic potential of

Fusarium species (Nelson *et al.*, 1990), microconidia was produced in long chains in *F. moniliforme* and short to medium length in *F. nygamai* and *F. napiforme*. *F. beomiforme* was the only species to produce an abundance of large globose microconidia which was the characteristic feature of this species and distinguished it from *F. nygamai*. Linear growth was the greatest at 25℃ for *F. oxysporum*, *F. moniliforme*, *F. nygamai* and *F. napiforme* except *F. beomiforme* for which maximum linear growth occured at 30℃.

Since in a chemotaxonomic study such as comparing the two-dimensional polypeptide patterns the protein looked as gene products, it indirectly revealed genetic relationships. Organism which showed numerous differences in polypeptides was also quite different genetically and had relatively stable distingushing morphological characteristics (Kawchuk *et al.* 1987).

In conclusion, it was supposed that *F. nygamai* and *F. napiforme* would be independent intersection which would lie between section *Liseola* and section *Elegans*, and that *F. beomiforme* might belong to section *Elegans*. The results of this study were consistent with previous studies about the comparison of isoenzyme patterns in *Fusarium* (Min and Kweon, 1994). For the accurate taxonomic position of these species, *F. nygamai*, *F. napiforme* and *F. beomiforme*, the studies on the electrophoretic karyotypes and RFLP of *Fusarium* are going on.

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