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A Chemotaxonomic Study on Geographical Variations of Korean Fucales Plants

3. Total lipid analysis by 3-dimensional TLC

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韓國產 모자반目 식물의 地理的 變異體에 대한 生化學的 分類

3. 三次元的 TLC에 의한 總脂質의 分析

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ABSTRACTS

The Fucales plants have very homogenous taxonomic characteristics at order level, but show many morphological varitations at species or subspecies levels. This study is one of the serial works to obtain more taxonomic data than morphotaxonomic ones and to clarify the taxonomic ranks and characteristics of Korean Fucales plants through physiological and biochemical analyses.

Total lipid compositions of Korean Fucales plants were analyzed by the 3-dimensional TLC method. Major components of phospholipids (PA, PC, PG, PE, DPG, LPE, LPC), neutral fat TG and sterols were commonly contained in each species. Unknown lipids X1, X2 and X3 were contained in genus *Sargassum*, but *Hizikia* was lacking X3 and *Myagropsis* was lacking both X2 and X3. The latter contained unique X8. From the view-point of the phylogeny of lipid metabolism, *Sargassum* might be more closely related to *Hizikia* than to *Myagropsis*. A variant of *S. patens* from Seongsan showed minute metabolic difference from that of the typical plant; but a variant of *S. serratifolium* did not show any significant difference from that of the typical plant.

INTRODUCTION

The compositions of fatty acids, sterols, steryl esters and some other lipids are sometimes useful taxonomic characteristics to help understanding phylogenetic relationships (Miller, 1962; Jacob, 1979; Matsushiro and Urzua, 1984; Cerri *et al.*, 1985; Arasaki *et al.*, 1986; Bergquist *et al.*, 1986). Especially in microbial taxonomy, fatty acid composition has proved useful in the classification of certain taxa (Lechevalier, 1977). For example, the degree of saturation and number of carbons in the fatty acid pattern may be characteristic for a particular taxon.

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Moreover, another class of lipids with recognized chemotaxonomical potential are the polar lipids such as phospholipids, glycolipids, and other lipids such as hopanoids, hydrocarbons and cartenoids (Shaw, 1975; Czeczuga, 1986).

Form this viewpoint, we tried to examine the total lipid compositions of Korean Fucales plants. Fucales is an unique, homogenous taxon of Phaeophyta, but shows many morphological variations at specific and sub-specific levels according to environmental conditions and geographical distributions (Fritsch, 1945; Yoshida, 1983; Bold and Wynne, 1985). After the morpho-taxonomical study on Korean Fucales (Yoo, 1975), several chemo-taxonomical studies were carried out (Yoo and Lee, 1988; Yoo *et al.*, 1988) to overcome the problematic polymorphic characteristics of this taxon. Now in this paper we report the different patterns and compositions of the total lipids (polar lipids, neutral lipids, and sterols, etc.) in Korean Fucales plants using 3-dimensional thin layer chromatography.

MATERIALS AND METHODS

The Korean Fucales plants were collected from Cheju Island on July 21–26, 1987. The sampling sites and method were described by Yoo and Lee (1988). Yoo and Lee (1988) also should be referred to for the list of collected Korean Fucales plants and their variants. For the separation of total lipids of Fucales plants, we used the 3-dimensional TLC method previously described by Kramer *et al.* (1983). This method has many advantages, for example, all lipid classes can be resolved quickly on one TLC plate and the relative ratio between lipid classes is preserved without loss of any lipid components.

RESULTS AND DISCUSSION

11 kinds of Korean Fucales plants(3 genera, 7 species, 4 variants) were subjected to total lipid analysis by 3-dimensional TLC. The results are shown in Figures 1–3 and summarized on Table 1. X1–X11 in Figures mean unknown lipid compounds, most of them presumed to be glycolipids and phospholipid derivatives. Also, C1 and C2 mean unknown sterol derivatives.

Major components of phospholipids(PA, PC, PI, PG, PE, DPG, LPE, and LPC, etc.) were present in all Korean Fucales plants even though their amounts were more or less different. Neutral fat TG and sterols(CE) were also contained similarily in each species. But there were many differences in compositions of unknown lipid compounds (X1–X2, C1, C2), FFA,DG, and LPC at generic level, at species level, even at geographical variant level (Table 1).

Unknown X1, X2 and X3 were found in many of the Korean Fucales plants. Among them X2 was present in all genera. But *Hizikia* was lacking X3 and *Myagropsis* was lacking both X1 and X3 (Fig. 1). X3 was found only in genus *Sargassum*. Genus *Myagropsis* contained unknown X8, as did *Sargassum patens* (sample no.4-A & B). *M. yendoi* (sample no.2B) also uniquely contained many unknown lipids (X5, X6, X7, X9, X10).

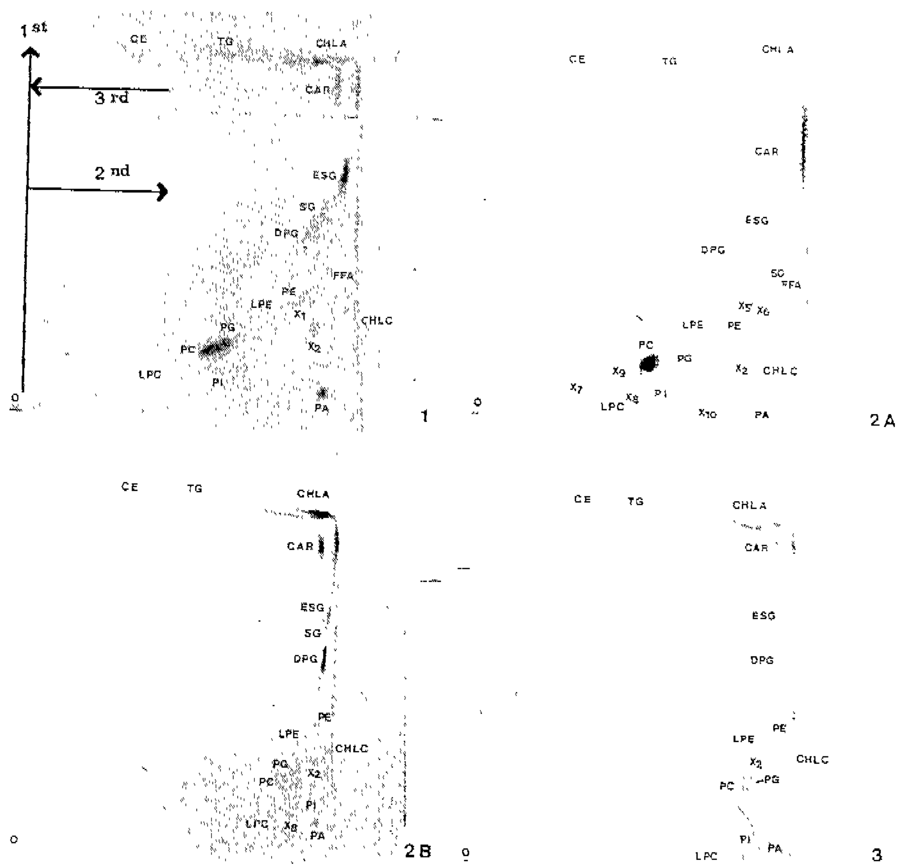

Fig. 1. 3-Dimensional TLC chromatograms for total lipid separation in *Hizikia fusiformis (1)*, *Myagropsis yendio*(2-A). *M. myagroides*(2-B), *Sargassum piluliferum*(3). Three-directional TLC separation of total lipids was performed on precoated Silica Gel G (Sigma, Co.). 20cm × 20cm and 250um in thickness. Direction of development, see Figure 1–1. Solvents: 1st direction, chloroform/methanol/28% aqueous ammonia (65 : 25 : 5) ; 2nd direction, chloroform/acetone/methanol/aectic acid/water (50 : 20 : 10 : 15 : 5) ; 3rd direction, hexane/diethyl ether/aectic acid (85:15:1). After 2nd development, lipid classes on the TLC plate were visualaized under UV light after spraying the plate with 0.1% solution of 2, 7-dichlorofluorescein in methanol. With removal of the adsorbent above the phospholipid region, only the neutral lipids were resolved in the third direction. After 3rd development, all spots were visualized by charring the plate following spraying with sulfuric acid/ethanol. (1:1, v/v). C1, C2, cholesterol derivatives; Car, carotenoid; CHLA, CHLC, chlorophyll a or c; CE, chloresterol ester; DG, diglyceride; DPG, diphosphatidyl glycerol; ESG, esterified steryl glucoside; FFA, free fatty acid; LPC, lysophosphatidyl choline; LPE, lysophosphatidyl ethanolamine; O, origin; PA, phosphatidic acid; PC, phosphatidyl choline; PE, phosphatidyl ethanolamine; PG, phosphatidyl glycerol;PI, phosphatidyl inositol; S, sterol; SG, steryl glucoside; TG, triglyceride; X1 ··· X11, unknown lipids.

S. patens (4A, 4B, 4C) had distinct unknown lipids, X8 and X11. Among them X11 was detected only from the geographical variant (4-B). A sprout of *S. patens* (4-C) did not contain X8 (Fig. 2).

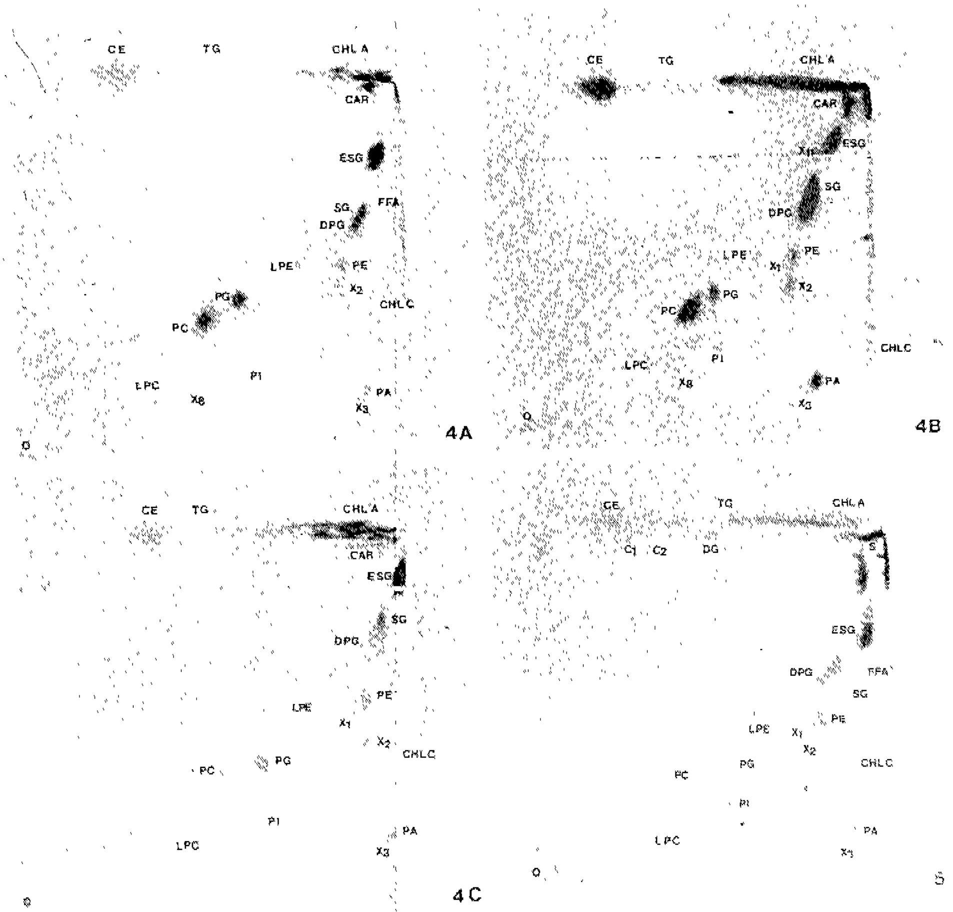

Fig. 2. 3-Dimensional TLC chromatograms for total lipid separation in *Sargassum patens*(4-A, B, C) and *Sargassum ringgoldianum*(5).

S. ringgoldianum (5) contained unidentified cholesterol derivatives C1 and C2, which were not detected in the other Fucales species (Fig. 2). Moreover, *S. ringgolidianum* had another distinct neutral fat (DG). Both *S. serratifolium* (6-A) and its variant (6-B) included X4, but the latter contained free fatty acid (FFA) which was different from the former. *S. giganteifolium* (13) was lacking X3 (Fig. 3) and *S. piluliferum* was also lacking X1 and X3 (Fig. 1).

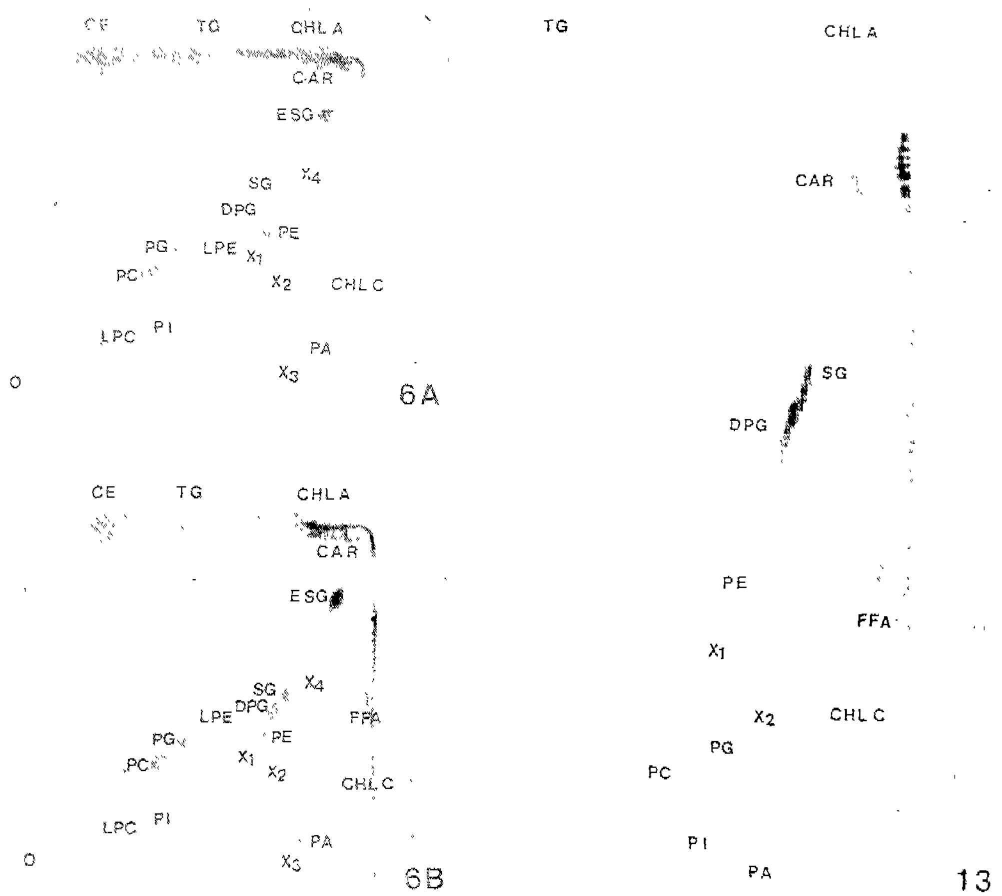

Fig. 3. 3-Dimensional TLC chromatograms for total lipid separation in *Sargassum serratifolium* (6-A, B), and *Sargassum giganteifolium* (13).

Although it should be noted that the fatty acid patterns of certain organisms may be influenced by lots of factors. *i.e.*, composition of growth medium, temperature, age, and techniques employed to analyze a sample (Jones and Krieg, 1984), samples in this experiment were collected from almost the same geographical region, in the same season, at the same temperature, etc., meaning that physico-chemical environmental factors were nearly the same. Therefore we can recognize several important things from our experimental results.

The phylogenetic relationship between *Sargassum* and *Hizikia* is closer than that between *Sargassum* and *Myagropsis*. *Hizikia* and *Myagropsis* are rather distantly related. *M. yendoi* was separated clearly form *M. myagroides* in biochemical metabolic aspect. A variant of *S. patens* growing in Seongsan, Cheju Isl. showed minute metabolic difference from that of a typical plant; but a variant of *S. serratifolium* did not show any significant difference from that of a typical

Table 1. Comparative scheme of unknown lipid components contained in Korean Fucales plants

Sample No.	Species	Unidentified and /or Distinct Lipid															
		X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11	C1	C2	FFA	DG	LPC
1–A	*Hizikia fusiformis*	+	+												+		+
2–A	*Myagropsis yendio*		+			+	+	+	+	+	+				+		+
2–B	*Myagropsis myagroides*		+						+						+		+
3	*Sargassum piluliferum*		+												+		+
4–A	*Sargassum patens*	+	+	+					+						+		+
4–B	*Sargassum patens*	+	+	+					+			+					+
4–C	*Sargassum patnes*	+	+	+													+
5	*Sargassum ringgolidianum*	+	+	+									+	+		+	+
6–A	Sargassum serratifolium	+	+	+	+												+
6–B	*Sargassum serratifolium*	+	+	+	+										+		+
13	*Sargassum giganteifolium*	+	+												+		

plant. *S. giganteifolium* and *S. piluliferum* were lacking X1 and X3, which were common to genus *Sargassum*.

All of the unknown lipid compounds should be identified in our further work, because chemical differences may also be the result of genetic variables (Howard, *et al*, 1980).

摘　　要

모자반目 식물은 目의 수준에서는 명확한 분류학적 특징을 갖지만 種 및 種 이하의 수준에서는 형태적 변이가 매우 큰 분류군이다. 본연구는우리나라에 생육하는 모자반目 식물을 생리·생화학적으로 분석하여 형태분류학적 특징 이외의 분류학적 자료를 모아 각 분류군의 지위 및 특성을 명확히 규명하려는일련의 연구중 하나이다.

모자반目 식물의 總脂質은 三次元的 TLC에 의하여 분석하였다. 각 種은 인지질(PA, PC, PI, PG, PE, DPG, LPE, LPC), 중성지방 TG와 스테롤을 공통으로 함유하였다. 모자반屬은 미지의 지질 X1, X2, X3를 공통으로 함유하였으며, 톳屬은 그중 X1과 X2를, 외톨개모자반屬은 X2만을 함유하였고 X8도 함유하였다. 이는 지질대사라는 계통 측면에서 모자반屬과 톳屬이 모자반屬과 외톨개모자반屬 보다 가깝다는 것을 시사해준다. 성산에서 채집된 쌍발이모자반 변이체는 원종과 다른 미지의 지질(X11)을 함유하여 지질대사의 차이를 보였으나, 톱니모자반의 변이체는 원종이 함유한 지질과 중요한 차이를 보이지 않았다.

REFERENCES

Arasaki, S., T. Sakurai, A. Kawakuchi and N. Murata. 1986. Lipid and fatty acid composition in red algae *Porphyra yezoensis*. *Jap. J. Phycol.* **34:** 94–100.

Bergquist, P.R., A. Laivs and R.C. Cambie. 1986. Sterol composition and classification of the Porifera. *Biochem. Syst. Ecol.* **14:** 105–112.

Bold, H.C. and M.J. Wynne. 1985. Introduction to the algae (2nd ed.) Prentice-Hall, N.J. 720 pp.

Cerri, R., F.De Simone, A. Dini and E. Ramundo. 1985. Sterols, steryl esters and fatty acids in some *Viccia faba* seeds. *Biochem. Syst. Ecol.* **13:** 11–13.

Czeczuga, B. 1986. Carotenoids in Gymnosperms. *Biochem. Syst. Ecol.* **14:** 13-15.

Fritsch, F.E. 1945. The structure and reproduction of algae. Vol. Ⅱ. Cambridge Univ. Press. Cambridge. 939 pp.

Howard, B.M., A.M. Nonomura and W. Fenical. 1980. Chemotaxonomy in marine algae: Secondary metabolite synthesis by *Laurencia* in unialgal culture. *Biochem. Syst. Ecol.* **8:** 329–336.

Jacob, J. 1979. Chemotaxonomic investigations on the cuticular lipids of beetles. *Biochem. Syst. Ecol.* **7:** 141–145.

Jones, D. annd N.R. Krieg. 1984. Serology and chemotaxonomy. *In,* Bergy's Manual of Systematic Bacteriology, Wiliams & Wiliams Co., Vol. 1, pp. 16–17.

Kramer, J.K.G., R.C. Fouchard and E.R. Farnworth. 1983. A complete separation of lipids by three-directional thin layer chromatography. *Lipids* **18:** 896–899.

Lechvalier, M.P. 1977. Lipids in bacterial taxonomy-a taxonomist's view. *Crit. Rev. Microbiol.* **5:** 109–210.

Matushiro, B. and A. Urzua. 1984. Sterols of some Chilean algae. *Biochem. Syst. Ecol.* **12:** 145–147.

Miller, J.D.A. 1962. Fats and Steroids. *In,* Physiology and Biochemistry of Algae, R.A. Lewin (ed.). Academic Press Inc. pp. 357–383.

Shaw, N. 1975. Bacterial glycolipids and glycophospholipids. *Adv. Microb. Physiol.* **12:** 141–167.

Yoo, S.A. 1975. Some members of Fucales (Phaeophyta) in Korea. MS thesis, SNU. 145 pp, 26 pls.

Yoo, S.A. and K.S. Lee. 1988. A chemotaxonomic study on geographical variations of Korean Fucales plants. 1. The analyses of pigment, phospholipid, neutral fat and inorganic polyphosphate. *Korean J. Phycol.* **3**(1): (in press).

Yoo, S.A., K.S. Park and K.S.Lee. 1988. A chemotaxonomic study on geographical variations of Korean Fucales plants. 2. Lectin. *Korean J. Phycol.* **3**(1): (in press).

Yoshida, T. 1983. Japanese species of *Sargassum* subgenus *Bactrophycus* (Phaeophyta, Fucales). *Journ. Fac. Sci., Hokkaido Univ.* ser. V (Botany), **13**(2): 99–246.

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