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Culture Collection of Marine Microalgae*

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해양 微細藻類의 배양수집

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ABSTRACT

The cultivation of marine microalgae as a live feed is essential for the seedling production of marine animals. It has some technical problems. Of these, the isolation and maintenance of the pure strains of microalgae from the nature are difficult for general mariculturists and researchers. To meet these problems, it needs to establish the culture collection of the microalgae in order to supply the strains to the demanders.

In this research, 80 strains of microalgae were isolated from the coastal water of Korea by the methods of capillary pipette, plating on agar and dillution. A culture collection was established in the Department of Aquaculture, National Fisheries University of Pusan. The 117 strains of the microalgae maintained in the culture collection will be supplied to the demanders without any difficulties.

요 약

해산동물의 종묘생산시 초기단계의 먹이로써 미세조류의 배양은 매우 중요하다. 그러나 자연으로부터 먹이생물로써 필요한 미세조류를 순수분리하여 순종을 보관하는 일은 일반 양식업자나 이 분야에 종사하지 않는 연구자에게는 매우 어려운 일이다. 따라서 미세조류를 필요로 하는 사람들에게 순종의 미세조류 strain을 공급할 수 있는 은행의 설립이 필요하다. 본 연구에서는 capillary pipette법, 한천도말법, 희석법을 이용하여 한국연안에서 80종의 미세조류를 분리하였다. 현재 부산수산대학교 양식학과 실험실에서 확보 배양중인 117종의 미세조류의 배양현황을 보고하였다.

Introduction

As the phytoplankton is the primary producer in the ocean, its role is very important for marine food chain. Recently, cultivation of phytoplankton draws a particular attention for various purposes.

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The early research on the cultivation of phytoplankton was focused on fundamental phycology to increase basic knowledge on living system. Most early attempts of phytoplankton culture derived from early experiments of Miquel(1892), who successed first in growing a few diatoms under artificial conditions. However, the active cultivation started from 1910's. Pringsheim (1912) used first soil extract as an enrichment to stimulate growth of phytoplankton, and many other investigators such as Allen (1914), Schreiber (1927), Provasoli et al. (1957), Guillard and Ryther (1962), Ukeles (1971) and Starr (1978) have innovated the culture technique.

Application of the potential of phytoplankton as a food supplement for humans in the daily diet (Becker 1988) and other cultivated animal as a live feed(Loosanoff and Davis 1963 ; Ukeles 1971) in aquaculture has been evaluated rapidly. In addition to these applications, the successful cultivation of microalgae further provides the materials for industrial and medical use (Hutner 1964 ; Carlucci and Bowes 1970). the test oraganisms for biossay (Skulberg 1970 ; Smayda 1970), and water quality assessment (Aleem 1970), the method of gas exchange for air revitalization in submarines and space crafts (Hannan and Patouillet 1963 ; Ammann and Fraser-Smith 1968) and waste water treatment (Hugnenin 1974), etc. Because of important availability of micoalgae mentioned above, the establishment of culture collection was necessary in order to the supply the strains to interested researchers and industrial institutions.

The Professor Dr. E. G. Pringsheim established the first collection of microalgae in Prague Czechoslovakia in the 1920's and later went to Cambridge where he established the collection which is now known as the Culture Collection of Algae and Protozoa (CCAP) at Cumbria and Oban (U. K).

While most collections are more concentrated in freshwater species rather than marine species, many smaller collections exist for marine algae. Besides of CCAP, the big collections are established in University of Texas (Austin), Bigelow Laboratory for Ocean Sciences (Maine) and American Type Culture Collection (Rockville) in the USA. Tropical and subtropical algae strains are maintained in Scripps Institution of Oceanography (California) and Solar Energy Research Institute (Colorado). The marine microalgae strains as the live feed for bivalve larvae are collected in Milford Laboratory of Northeast Fisheries Center (Connecticut), and heterotrophic microalgae are maintained at Martec Corporation in Maryland (Gladue 1991).

In Korea, while on marine phytoplankton researchs are carried out in varions aspects, its studies through cultivation are few. In particular, the study on isolation and purification of microalgae is not yet dealt. However, considering the importance of aquatic sciences and its related industry with marine microalgae, the establishment of culture collection of marine microalgae in Korea is urgent.

The aim of this research is to introduce the methods for isolation and purification of marine microalgae and the list of culture collection carried out recently in the live feed laboratory of the Department of Aquaculture, National Fisheries University of Pusan (NFUP).

Isolation and Purification

The natural marine microalgae were mainly collected by netting and surface water sampling in nearshore areas, tidal pools, salt ponds and estuaries. Microalgae were isolated from crude samples

that have been brought into the laboratory. Depending on density of samples, the sample are concentrated by centrification or diluted by filtered sea water. If the crude samples are of patch or contaminated with fouling organism, the ultrasonic apparatus is used to separate the individual microalgal cells before isolation.

I adopted three isolation techniques ; direct captures by capillary pipette under stero and compound microscope, streak plating on agar media and dilution. Isolation technique depends on the algal type, size and diversity of crude sample. Larger thalli, such as filaments, can be isolatated by the capillary pipette method. However, microalgae less than 10 μm are difficult to see and to isolate with the capillary pipette under microscopes. In this case, the method of streak plating on agar is more convenient for isolation. On the other hand, crude samples with low diversity collected from the polluted or red tide areas are easy to isolate by the continuous dilution method.

The direct capture method described by Howhaw and Rosowski (1963) is adopted for this study. The capillary pipette is made with commercial pasteur type pipette (length 20 cm, diameter of narrow end 2 mm) under a low or pilot flame of a burner. A single inverted plastic petri dish top is used as an isolation dish. Several drops of natural collection are placed in the center of an inverted plastic petri dish and 4 drops of filtered sea water are placed in four positions encircling the natural collection. Using a sterile capillary pipette, the disired microalgal units are transferred by the capillary pipette from the natural collection to one of the four drops of filtered sea water. The transfers are repeated as soon as possible until only a single cell is left in a drop. Finally, this single cell is again transferred to round cover glass (diameter 9 mm) resting on a slide glass, and then examined with the compound microscope. This small cover glass with a single cell is then transferred to 10 mℓ of the f/2 liquid culture medium (Guillard and Ryther 1962) in a test tube, and incubated under suitable growth condition.

When microalgae units are less than 10 μm in size, plating method described by Hoshaw and Rosowski (1963) is also adapted. The glass petri dish (90×15 mm) contains LDM agar (Starr 1978) as the growth media with the 1/2 depth of the dish. One drop of natural collection is placed near the periphery of the agar, and the parallel streaks of the suspension on the agar are made as aseptically using sterile loop as microbiological technique. The dish is coverted and incubated under suitable growth condition. After 2−3 week's incubation, the desired colonies are removed using a fine capillary pipette, and placed in a drop of f/2 medium on a slide glass. After the observation of monospecies colony with the compound microscope, this colony is transferred to f/2 liquid medium in a test tube.

Even axenic culture cannot be produced by the dilution method, this technique is particularly useful when the starting material is preponderantly unialgal such as in polluted or red tide areas. The principle is to dilute a sample containing the wanted microalgae with medium. The multi cell culture chamber (vol. 1 mℓ) is more convenient than the test tube to dilute the sample for this method. Throndsen (1978) and Belcher and Swale (1982) give a more detailed account of the dilution method.

The monoxenic microalgae culture needs sometimes to be purified without bacteria for the purpose of physiological, nutritional and biochemical studies of microalgae. The purification can be possible by the successive plating method or repeated transfer of microalgae to new sterile medium with capillary pipettes. If the contaminatnts adhere to the algal units, the ultrasonic vibration may be useful to seperate the contaminants physically. For the microalgae with tenaciously attached contami-

nants, it may be necessary to kill or inhibit growth of the contaminants by antibiotics or potassium tellurite treatment. However, in general, as bacteria in culture media provide vitamin and CO_2 to microalgae (Schmitt 1965), the presence of bacteria is regarded as a food source in microalgae culture (Asher and Spalding 1982).

Culture Media

In the early days of phytoplankton culturing studies, growth media consisted of seawater nutrient as nitrate, phosphate and silicate. But, Fφyn (1934) improved marine culture media by the addition of Pringsheim's soil extract. Fφyn's Erdschreiber medium has probably been the most extensively used as a marine medium, resulting in the successful culture of many species of microalgae (Ukeles 1976). In 1950's, with the discovery that many algae needed vitamin and heavy metal for growth (Provasoli et al. 1957, Provasoli 1958), the number of marine algae could be cultured axenically, and various kinds of marine media were improved.

In practice, the medium composition should be simple and easy to prepare. In this respect, f/2 medium (Guillard and Ryther 1962) as liquid medium and LDM agar (Starr 1978) as solidified agar medium were used in this study. In addition, Fφyn's Erdschreiber medium (Fφyn 1934), proteous agar(Starr 1978), S. K. medium (Sorkin and Kraus 1958) and SOT medium (Ogawa and Terui 1972) were also used partially for culture collection.

The composition of the medium used in the study are listed in Table 1.

Culture Facility

Microalgae culture needs a light source, for which daylight may be adequate. A work-in-culture room (3×3×2.5 m) was located near the north-facing window to avoid direct sunlight. For the purpose of the control of light intensity and photoperiod, the electronic fluorescent tubes (40 W) were installed under each stainless shelf. At the beginning of culture, 20−22 ℃ temperature and continuous illumination with ca. 5,000 lux with 24hr day length maintained. But, when the growth of microalgae attained to stationary phase, low temperature (16−17 ℃) and light illumination was (ca, 3000 lux with 12hr day length) were maintained.

A cotton plug without aeration of culture flask (250 mℓ) was used for the stock culture of strains. The culture strains were maintained by the routine subculture. Depending on the strains, some diatom required subculturing every 2−3 week, while green and blue green algae persist for 2−3 months in a healthy state.

A full list of microalgae strains maintained in the laboratory is shown in Table 2.

Up to now, 122 strains of the collection have been supplied free of charge to domerstic and foreign researchers, and aquaculturists, and this supply will be continued.

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Table 1. Growth media used for isolation and subculture of the microalgae in the collection

LDM agar (Starr 1978)	
Bristol's solution	100 mℓ
Biotin	0.25 μg
Vitamin B_{12}	0.15 μg
PIV metals	6 mℓ
Trypton	1 g
Agar	12 g
Seawater	900 mℓ
Proteose agar (Starr 1978)	
Bristol's solution	1000 mℓ
Proteose peptane	1.0 g
Agar	15.0 g
Bristol's solution (Bold 1949)	
$NaNO_3$	250 mg
$CaCl_2$	25 mg
$MgSO_4 \cdot 7H_2O$	75 mg
K_2HPO_4	75 mg
$NaCl_2$	250 mg
Distilled water	1000 mℓ
A drop of 1.0% $FeCl_3$ solution	
PIV metal solution (Provasoli and Pintner 1959)	
$FeCl_3 \cdot 6H_2O$	97 μg
$MnCl_2 \cdot 4H_2O$	41 μg
$ZnCl_2$	5 μg
$CoCl_2 \cdot 6H_2O$	2 μg
Na_2MoO_4	4 μg
Na_2EDTA	0.750 mg
Distilled water	1000 mℓ
f/2 medium (Guillard and Ryther 1962)	
$NaNO_3$	150 mg
NaH_2PO_4	8.69 mg
Fe-EDTA	10 mg
$MnCl_2$	0.22 mg
$CoCl_2$	0.11 mg
$CuSO_4 \cdot 5H_2O$	0.0196 mg
$ZnSO_4 \cdot 7H_2O$	0.044 mg
$Na_2SiO_3 \cdot 9H_2O$	30−60 mg
$Na_2MoO_4 \cdot 2H_2O$	0.012 mg
B_{12}	1.0 μg
Biotin	1.0 μg
Thiamine. HCl	0.2 mg
Sea water	1000 mℓ
Erdschreiber medium (Føyn 1934)	
$NaNO_3$	100 mg
$Na_2HPO_4 \cdot 12H_2O$	200 mg
Soil extract	50 mℓ
Sea water	1000 mℓ
SOT medium (*Spirulina platensis*, Ogawa and Terui 1970)	
$NaHCO_3$	16.8 g
K_2HPO_4	0.5 g
$NaNO_3$	2.5 g
K_2SO_4	1.0 g
NaCl	1.0 g
$MgSO_4 \cdot 7H_2O$	0.2 g
$CaCl_2 \cdot 2H_2O$	0.04 g
$FeSO_4 \cdot 7H_2O$	0.01 g
$Na_2EDTA \cdot 2H_2O$	0.08 g
Na3-versenol	3.0 mg
Fe(as Cl^-)	0.2 mg
$B(H_3BO_3)$	0.2 mg
Mn(as Cl^-)	0.1 mg
Zn(as Cl^-)	0.05 mg
Co(as Cl^-)	1.0 μg
$MO(Na_2MoO_4)$	0.05 μg
Cu(as Cl^-)	2.0 μg
Distilled Water	1000 mℓ
S. K. medium (Sorkin and Krauss 1958)	
KNO_3	1.25 g
KH_2PO_4	1.25 g
$MgSO_4 \cdot 7H_2O$	1.00 g
$CaCl_2$	0.084 g
$FeSo_4 \cdot 7H_2O$	0.05 g
Krauss metal sol.*	0.1 mℓ
Distilled water	1000 mℓ
*Krauss metal solution	
H_3BO_3	2.86 g
$MnCl_2 \cdot 4H_2O$	1.81 g
$ZnSO_4 \cdot 7H_2O$	0.022 g
$CuSO_4 \cdot 5H_2O$	0.079 g
MoO_3	0.015 g
$Ca(NO_3)_2 \cdot 4H_2O$	59.0 g
$CoCl_2 \cdot 6H_2O$	0.04 g
Distilled water	1000 mℓ
HCl	5 drops

Table 2. List of microalgae strains in the collection (A=Axenic on agar slope ; B=Bacteria present ; L=Liquid medium (Axenic) ; LB=Liquid+bacteria only ; M1=Erdschreiber media ; M2=f/2 media ; M3=SK media ; M4=SOT media ; M5=LDM media ; M6=Proteous media ; CP=Capillary pipette method ; DM=Dilution method ; PA=Plating on agar ; CCAP=Culture Collection of Algae and Protozoa, U. K. ; NFUP=National Fisheries University of Pusan ; SERI=Solar Energy Research Institute, USA ; UD=University of Delaware, USA ; UTEX=University of Texas, USA)

NO.	Species	NFUP NO.	Medium	State	Korean Strains			Foreign Strains	
					Source	Isolation Method	Date	Deposition	Date of Receipt
1	*Amphora normanii*	64	M2, M5	L, LB	Haewundae	PA	1986		
2	*Amphora perpusilla*	51	M2, M5	A, LB	Incheon	PA	1986		
3	*Amphora* sp.	29	M2, M5	A				SERI/Ampho-1	1986
4	*Amphora* sp.	97	M2	LB	Mokpo	PA	1986		
5	*Amphora* sp.	106	M2	LB	Incheon	DM	1986		
6	*Aphora* sp.	110−1	M2	LB	Haewundae	CP	1990		
7	*Amphora* sp.	110−2	M2	LB	Haewundae	CP	1990		
8	*Amphora* sp.	110−3	M2	LB	Haewundae	CP	1990		
9	*Amphora* sp.	110−4	M2	LB	Haewundae	CP	1990		
10	*Amphora* sp.	110−5	M2	LB	Haewundae	CP	1990		
11	*Amphora* sp.	110−6	M2	LB	Haewundae	CP	1990		
12	*Amphora* sp.	110−7	M2	LB	Haewundae	CP	1990		
13	*Amphora* sp.	110−8	M2	LB	Haewundae	CP	1990		
14	*Amphora venata*	54	M2	L, LB	Suncheon	CP	1986		
15	*Anabaena* sp.	56	M2, M5	L, LB	?	?	?		
16	*Anabaena* sp.	56−1	M2, M5	L, LB	?	?	?		
17	*Caloneise schroden*	80	M3, M6	A, LB	Incheon	PA	1986		
18	*Chaetoceros gracilis*	6	M2, M5	L, LB				UD	1986
19	*Chaetoceros simplex*	8	M2, M5	LB				Japan	1986
20	*Chaetoceros* sp.	75	M2	L, LB	Incheon	PA	1986		
21	*Chlamydomanas moewusii*	43	M3, M6	A, LB				UTEX 91	1984
22	*Chroomonas* sp.	19	M2, M5	A, LB	Nacdong	DM	1985		
23	*Chroomonas* sp.	18	M2, M5	A, LB	Nacdong	PA	1985		
24	*Chroomonas* sp.	17	M2, M5	A, LB				UTEX LB 2000	1985

Table 2. (Continued)

25	*Chlorella ellipsoidea*	27	M2, M5	L, LB				Japan	1985
26	*Chlorella ellipsoidea*	37	M3, M6	A, LB				UTEX 247	1985
27	*Chlorella ellipsoidea*	38	M3, M6	A, LB				UTEX 20	1985
28	*Chlorella protothecoides*	39	M3, M6	A, LB				UTEX 25	1985
29	*Chlorella pyrenoidosa*	36	M3, M6	A, LB				UTEX 26	1985
30	*Chlorella stigmatophora*	26	M1, M2	L, LB				UTEX 993	1985
31	*Chlorella vulgaris*	52	M2	L, LB	Nacdong	DM	1986		
32	*Chlorella vulgaris*	52−1	M3	L, LB	Nacdong	DM	1987		
33	*Chlorella vulgaris*	35	M3, M	A, LB				UTEX 259	1985
34	*Chlorella* sp.	107	M2	LB	Kamcheon	DM	1988		
35	*Chlorella* sp.	109−1	M2	LB	Suncheon	CP	1990		
36	*Chlorella* sp.	109−2	M2	LB	Suncheon	CP	1990		
37	*Chlorella* sp.	109−3	M2	LB	Suncheon	CP	1990		
38	*Chlorella* sp.	111	M2	LB	Yeocheon	DM	1990		
39	*Chlorella* sp.	113−1	M2	LB	Nacdong	DM	1990		
40	*Chlorella* sp.	113−2	M2	LB	Nacdong	DM	1990		
41	*Chlorella* sp.	113−3	M2	LB	Nacdong	DM	1990		
42	*Chlorella* sp.	114−1	M2	LB	Nacdong	DM	1990		
43	*Chlorella* sp.	114−2	M2	LB	Nacdong	DM	1990		
44	*Chlorella* sp.	114−3	M2	LB	Nacdong	DM	1990		
45	*Cryptomonas ovata*	44	M3, M6	A, LB				UTEX 358	1984
46	Cyanophyte	93	M2	L	Incheon	PA	1986		
47	Cyanophyte	98	M2	L	Incheon	DM	1986		
48	Cyanophyte	101	M2	L	Incheon	PA	1986		
49	*Cylindrotheca closterium*	59	M2	A, LB	Incheon	DM	1986		
50	*Cylindrotheca closterium*	68	M2	L, LB	Haewundai	CP	1986		
51	*Cylindrotheca closterium*	85	M2, M5	L, LB	Incheon	CP	1986		
52	*Cyclotella* sp.	13	M2, M5	A, LB				SERI-S/Cyclo	1986
53	*Cyclotella* sp.	9	M2, M5	LB	Haewundai	PA	1985		
54	*Cyclotella* sp.	81	M2	L, LB	Haewundai	PA	1986		
55	*Dunaliella tertiolecta*	25	M2, M5	A, LB	Incheon	PA	1985		
56	*Dunaliella tertiolecta*	92	M2	LB				Japan	1985

Table 2. (Continued)

57	*Euglena* sp.	74	M2	A, LB	Incheon	PA	1986		
58	*Eudorina elegans*	69	M2	A, LB	Chungmoo	PA	1986		
59	*Gloeocytis* sp.	62	M2	L, LB	Nacdong	DM	1986		
60	*Hantzschia marina*	67	M2, M5	LB	Incheon	CP	1986		
61	*Heterosigma* sp.	23	M2, M5	A, LB	Masan	PA	1985		
62	*Heterosigma* sp.	82	M2	L, LB	Incheon	CP	1986		
63	*Isochrysis* aff *galbana*	5	M2, M5	L, LB				UD	1986
64	*Isochrysis galbana*	14	M2	LB				Japan	1985
65	*Lynbya elegans*	70	M2, M5	A, LB	Mokpo	PA	1986		
66	*Leptocylindrus danicus*	78	M2, M5	L, LB	Nacdong	DM	1986		
67	*Microcystis aeruginosa*	49	M2, M5	L, LB	Nacdong	CP	1985		
68	*Microcystis aeruginosa*	58	M2, M5	A, LB	Chungmoo	PA	1986		
69	*Microcystis aeruginosa*	61	M2, M5	L, LB	Nacdong	PA	1986		
70	*Microcystis* sp.	73	M2, M5	A, LB	Masan	PA	1985		
71	*Monochrysis lutheri*	15	M2	LB				Japan	1985
72	*Monochrysis lutheri*	90	M2	LB				Seasalt Ltd.	1991
73	*Monoraphidium* sp.	32	M2, M5	A				SERI-S/Monor-2	1986
74	*Nannochloris oculata*	22	M2, M5	L, LB				UTEX LB 1998	1985
75	*Nannochloropsis salina*	24	M2, M5	L, LB				SERI-S/Nanno-2	1986
76	*Nannochloropsis* sp.	87	M2, M5	L				Israel	1988
77	*Navicula incerta.*	1	M2, M5	A, LB				UTEX 2046	1985
78	*Navicula incerta*	94	M2	L	Incheon	PA	1986		
79	*Navicula* sp.	76	M2, M5	A, LB	Incheon	PA	1986		
80	*Navicula* sp.	95	M2	L	Incheon	CP	1986		
81	*Navicula* sp.	102	M2	L	Incheon	PA	1986		
82	*Navicula* sp.	112	M2	L	Haeunnder	CP	1990		
83	*Nitzschia closterium*	12	M2, M5	LB	Nacdong	DM	1986		
84	*Nitzschia dissipata*	11	M2, M5	L, LB				SERI-S/Nites-2	1986
85	*Nitzschia* sp.	3	M2, M5	A, LB				Japan	1985
86	*Nitzschia obtusa* var *scalpelliformis*	55	M2	L, LB	Incheon	CP	1986		
87	*Nitzschia obtusa* var *scalpelliformis*	63	M2	L, LB	Incheon	CP	1986		
88	*Oocytis pusilla*	33	M3, M6	A				SERI-S/Phaeo-1	1986

Table 2. (Continued)

89	*Oscillatoria* sp.	50	M2, M5	A, LB	Chungmoo	PA	1986		
90	*Oscillatoria tenuis*	60	M2	A, LB	Incheon	PA	1986		
91	*Palmella mucosa*	71	M2	A, LB	Incheon	PA	1986		
92	*Phaeodactylum tricornutum*	2	M2, M5	A, LB				SERI-S/Phaeo-1	1986
93	*Phaeodactylum tricornutum*	10	M2, M5	A, LB				UTEX 2089	1984
94	*Phaeodactylum tricornutum*	7	M2, M5	LB				UD	1986
95	*Phaeodactylum tricornutum*	72	M2, M5	A, LB	Nacdong	PA	1985		
96	*Platymonas subcordiformis*	21−1	M2, M5	A, LB				UTEX 171	1985
97	*Rhaphoneis* sp.	53	M2	L, LB	Suncheon	CP	1986		
98	*Rhaphoneis* sp.	96	M2	L	Suncheon	CP	1986		
99	*Scenedesmus* sp.	79	M3	L, LB	NFUP Pond	CP	1986		
100	*Skeletonema costatum*	46	M2	L, LB	Masan	CP	1986		
101	*Skeletonema costatum*	47	M2	L, LB	Nacdong	CP	1985		
102	*Sticococus bacillaris*	16	M2, M5	A, LB				Japan	1985
103	*Sticococus* sp.	103	M2	L	Incheon	DM	1986		
104	*Sticococus* sp.	104	M2	L	Masan	PA	1985		
105	*Sticococus* sp.	108−1	M2	L	Kamcheon	CP	1988		
106	*Sticococus* sp.	108−2	M2	L	Kamcheon	CP	1988		
107	*Spirulina platensis*	88	M4	L				Oceanic Inst.,USA	1991
108	*Tetraselmis suecica*	20	M2, M5	A, LB				CCAP 66/22A	1985
109	*Tetraselmis tetrathele*	86	M2	L				Oceanographic Inst., USA	1988
110	*Tetraselmis* sp.	91	M2	L	Haewundae	DM	1991		
111	*Thalassiosira fluviatils*	4	M2, M5	L, LB				CCAP 1085/1	1985
112	*Thalassiosira pseudonanna*	89	M2	L				Sea Salter Ltd., U. K.	1991
113	*Thalassiosira wessflogii*	84	M2, M5	L, LB	Nacdong	DM	1986		
114	*Thalassiosira wessflogii*	65	M2, M5	LB	Incheon	PA	1986		
115	*Thalassiosira* sp.	99	M2	LB	Masan	DM	1986		
116	*Thalassiosira* sp.	100	M2	LB	Masan	DM	1986		
117	*Thalassiosira* sp.	105	M2	LB	Nacdong	CP	1986		

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