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Identification of *Chlorella* spp. isolates using ribosomal DNA sequences

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Abstract. Members of the *Chlorella* species are very simple unicellular algae, easy to cultivate and widely used in various physiological studies. Their morphological and physiological characteristics, however, normally change with the environment, making species identification difficult. To elucidate the relationship between various strains of *Chlorella*, this investigation analyzed the nuclear-encoded and chloroplast-encoded small-subunit rDNA sequences of four strains of *Chlorella* using PCR techniques. These strains were isolated from different rivers and ponds in Taiwan and Indonesia, and then compared and identified using stock strains of *Chlorella* spp. from the culture collection centers, and published DNA sequence data from Genbank. Experimental results attributed the isolated strains mainly to *C. sorokiniana*, a common species of green algae which grows in freshwater ecosystems at around 36°C. In addition, phylogenetic analysis of nuclear-encoded and chloroplast-encoded small-subunit rDNA sequences from spherical green algae of the genera *Chlorella* revealed the sequences to closely resemble each other. Further analyses indicated that *Chlorella* spp. 216 was close to *Chlorella* spp. 21 and I. Generally, the chloroplast data sets supported the lineages more than the nuclear data sets did. Strains 21 and 216 were closer to I. Comparisons with some of the morphological and biochemical data indicated that the phylogenetic analysis of rDNA sequences was in line with results obtained by conventional methods.

Keywords: *Chlorella*; Chloroplast; Nuclear; PCR; Phylogeny; Small subunit.

Introduction

The simple and common green algae of the genus *Chlorella* (Beijerinck, 1890) are placed below the order Chlorococcales and family Chlorellaceae (Hoek et al., 1995). Reproduction is asexual and achieved by producing non-motile autospores. Species of this genus are widespread in fresh water and in the sea, air, and soil. Warburg (1919) discovered that pure cultures of these fast-growing microorganisms can be used as the ideal experimental materials for research on photosynthesis, nitrate reduction, physiology and biochemistry. Recently, *Chlorella* have been extensively studied and employed in various practical applications in agriculture and biotechnology. *Chlorella* are also used as protein-rich foods for sewage oxidation (Kessler, 1982). However, their cells do not exhibit characteristics that differentiate them from the morphological properties which are typically the basis of a classical taxonomic treatment of other algae (Shihira and Krauss, 1965).

Although the traditional taxonomic characteristics of *Chlorella* spp. indicate that morphological, biochemical and physiological properties are used in its identification, the cell size and shape are variable and largely depend on varying age, nutrition, and environmental factors (Fott and Novakova, 1969; Komárek and Fott, 1983). Additionally, certain biochemical and physiological characteristics are not species specific (Kessler, 1982; Kessler, 1984; Kalina and Puncocharova, 1987). Therefore, classifying unknown isolated samples can be difficult.

The high number of copies and the inclusion of conserved and variable regions by evolution of ribosomal RNA genes (rDNA) have made possible a new method of species identification, classification and phylogenetic relationship determination (Mullis et al., 1986; Mullis and Faloona, 1987; Saiki et al., 1988). Algae cells have chloroplast genes just like higher plants, meaning cells have two varieties of SSU rDNA, nuclear-encoded and chloroplast-encoded. These characteristics have been considered for algae taxonomy and phylogenetic relationships (Huss and Sogin, 1990; Wilcox et al., 1992; Steinkötter et al., 1994; Schreiner, 1995). Meanwhile, Krienitz et al. (1996) compared the morphology and nuclear encoded SSU rDNA of green algae and found them closely related.

Recently, biochemical, physiological, and ultrastructural characters, together with molecular phylogeny based on the complete 18sRNA sequence, have led to the proposal that only four species should be kept in the genus *Chlorella*: *C. vulgaris*, *C. lobophora*, *C. sorokiniana*, *C. kessleri* (Huss et al., 1999).

Phylogenetic analysis of the order Chlamydomonadales revealed that this set of chloroplast data exhibited stronger support for comparable lineages than the set of nuclear data (Buchheim et al., 1996). Therefore, in this study field isolates from rivers in Indonesia and Taiwan are investigated, and the main observed cells were spherical,

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Table 1. Source of the *Chlorella* spp.

Strain number	Location	Ecological environment	
		pH	Temp (°C)
21	Wonokromo, Jagir, Surabaya, Indonesia	7.9	30
216	Wonokromo, Jagir, Surabaya, Indonesia	7.5	32
I	Ching-lung His, Ching-lung Village, Taimali Town, Taitung, Taiwan	7.2	27
V	Alun-alun, Malany, Indonesia	7.9	27

Table 2. Chlorellaceae rDNA sequence from GenBank (EMBL).

Chlorellaceae species	Source of SSU rDNA		
	Nuclear	Chloroplast	
Nanochlorum eucaryotum			
Strain Mainz 1	X06425		
Prototheca wickerhamii			
Strain 263-11	X56098	X74309	
Strain Pore 1283	X56099		
Prototheca zopfii			
Strain SAG 263-1a	X63519		
Chlorella species			
C. ellipsoidea			
Strain 211-1a	X63520		
Strain IAM C87	D13324	X12742	
C. emersonii			
Strain NIES 690	AJ242761 (NS12)	AJ242751 (CS12)	AJ242747 (CS34)
C. homosphaera			
Strain CCAP 211/8e	X73996		
C. kessleri			
Strain SAG 211-11g		X65099	
Strain SAG 211-11h		D11346	
Strain IAM C-208	AJ242765 (NS12)	AJ387750 (CS12)	AJ242769 (CS12)
C. lobophora			
Strain Andreyeva 750-I	X63504		
C. luteoviridis			
Strain CCAP 211/3	AJ242758 (NS12)	AJ242767 (CS12)	AJ242768 (CS34)
Strain SAG 211-2a	X73998		
C. mirabilis			
Strain Andreyeva 748-I	X74000	X65100	
C. pyrenoidosa			
Strain IAM C-101	AJ242762 (NS12)	AJ242752 (CS12)	AJ242749 (CS34)
C. protothecoidea			
Strain 211-7a		X65688	
C. saccharophila			
Strain 3.80		D11348	
Strain 211-1d		D11349	
Strain SAG 211-9a	X63505		
Chlorella sp. 21	AJ242760 (NS12)	AJ242754 (CS12)	AJ387748 (CS34)
Chlorella sp. 216	AJ242759 (NS12)	AJ387753 (CS12)	AJ387749 (CS34)
Chlorella sp. I	AJ242764 (NS12)	AJ238891 (CS12)	AJ387755 (CS34)
Chlorella sp. V	AJ242766 (NS12)	AJ387759 (CS12)	AJ387752 (CS34)
C. sorokiniana			
Strain 211-8k	X62441	X65689	
Strain Prag A14	X74001		
Strain IAM C-210	AJ242763 (NS12)	AJ387756 (CS12)	AJ387751 (CS34)
C. vulgaris			
Strain 211-11b		X16579	
Strain 211-1e		D11347	
Strain IAM 211/19	AJ242755 (NS12)	AJ242754 (CS12)	AJ242771 (CS34)
Strain NIES 227	AJ242756 (NS12)	AJ242750 (CS12)	AJ242770 (CS34)
Strain IAM C-27	AJ242757 (NS12)	AJ242753 (CS12)	AJ242748 (CS34)
C. zofingiensis			
Strain SAG 211-14a	X74004		
Strain Bethesda C-1.2.1	X74005		
Chlorella sp. SAG 11-18	X74006		

reproduced with autospores, and had no flagella. These basic characteristics suggest that isolates are primary and belong to *Chlorella*. Partial SSU rDNA data from nuclear regions are compared with the chloroplast of all isolates herein, and various other conventional identification methods are also examined.

Materials and Methods

Taxon Selection

Field samples of the investigated microalgae were collected from selected rivers and ponds in Taiwan and Indonesia. Table 1 lists some physicochemical and ecological data from these environments. Meanwhile, Table 2 lists the nuclear and chloroplast encoded SSU rDNA sequence data (accession numbers are given in parentheses) taken from the GenBank/EMBL databases and integrated into the analyses, including *Chlorella, Prototheca,* and *Nanochlorum,* three genera of Chlorellaceae.

Nuclear and Chloroplast Sequence Data

Both nuclear and chloroplast sequence data were obtained from the PCR amplifications using genomic DNA. Cells were cultivated in an autotrophic media at 25°C under continuous illumination. They were then harvested by centrifugation (10,000 rpm for 30 min) and ground in porcelain vessels with liquid nitrogen. Total cellular DNA was extracted from cell samples, as described by Hseu et al. (1996a). Table 3 illustrates PCR primers used for amplifying and sequencing the partial SSU segment. PCR amplification reactions were performed as described by Hseu et al. (1996a,b). For DNA sequence analysis, an ABI PRISM 377-96 DNA-sequencer (Perkin-Elmer, CA, USA), was used with ABI PRISM BigDye. Meanwhile, a terminator cycler sequencing ready reaction kit (PE Applied Biosystem, USA) was also employed.

Data Analyses

The alignment of nuclear and chloroplast sequences was conducted using SeqApp 1.9 (Gilbert, 1992). Phylogenetic analyses were performed via the SSU data set using the parsimony method with the program PAUP, Version 4.0 (Swofford, 1998). Bootstrap values from 100 repeated samplings were calculated for each set of data. All tree relationships were rooted through the outgroup method.

Conventional Methods

Isolated cell samples were observed under Nikon Eclipse E600 optical microscopes with a phase contrast device. Meanwhile, thermophilic ability was tested by cells grown on agar slants at elevated temperature. Finally, starch hydrolysis abilities were assessed for all isolates on agar plates containing suitable quantities of soluble starch.

Results

Structures of the Sequence Data

Table 4 summarizes the primary structure of the two independent data sets. This table also compares variable and informative sites, indicating that the chloroplast data are more variable than the nuclear data in terms of number and percentage of both variable and informative sites.

Phylogenetic Analysis of Nuclear Data

According to the primers designed by White et al. (1990), the SSU sequence from the GenBank/EMBL database was separated into several regions, namely the NS12, NS34, NS56, and NS78 regions. Different regions in parsimony analysis exhibit the same tendency (data not shown), so the NS12 region was employed herein to ana-

Table 3. Primers used in PCR reaction.

Primer	Source	Primer sequence	Product size (bp)
NS1	Nuclear SSU	5'-GTAGTCATATGCTTGTCTC-3'	
NS2	Nuclear SSU	5'-GGCTGCTGGCACCAGACTTGC-3'	550
CS1	Chloroplast SSU	5'-CGGCTGATTAGCTTGTTGG-3'	
CS2	Chloroplast SSU	5'-GAGTGCTTTCGCCTTTGG-3'	500
CS3	Chloroplast SSU	5'-AAGGCCAAAGCACTCTGC-3'	
CS4	Chloroplast SSU	5'-TTCCTCCGGCTTATCACC-3'	450-500

Table 4. Nucleotide sequence variation in the nuclear SSU NS12 region and in the chloroplast SSU CS14 region in Chlorellaceae.

Category of comparison	Nuclear Data	Chloroplast Data
Positions aligned	446	854
Chlorellaceae		
Total variable sites	129 (28.9%)	380 (44.5%)
Phylogenetically informative sites	41 (9.2%)	164 (19.2%)
Informative / variable	31.8%	43.1%
Chlorella spp.		
Total variable sites	73 (16.4%)	243 (28.5%)
Phylogenetically informative sites	23 (5.2%)	132 (15.5%)
Informative / variable	31.7%	54.4%

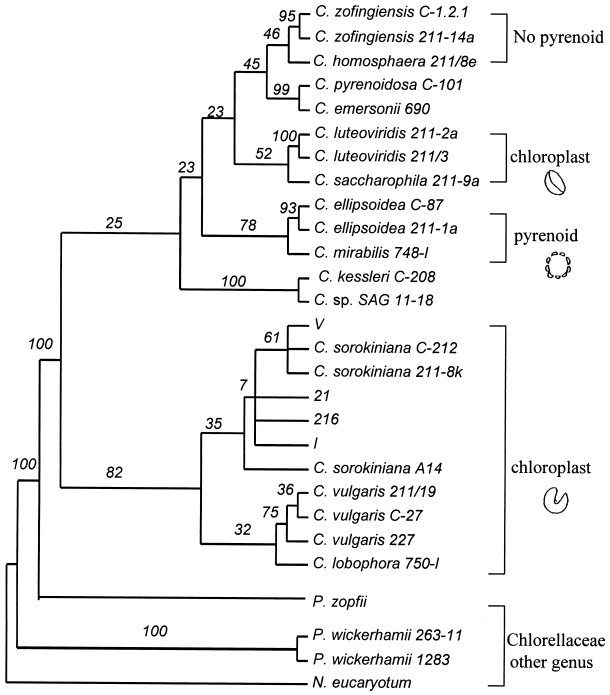

Figure 1. Phylogenetic relationships inferred by *Chlorella* spp. nuclear SSU NS12 nucleotide sequence data. The tree produced by use of a heuristic search in PAUP 4.0 from cladistically informative characters. Tree length = 355; consistency index (CI) = 0.6347; retention index (RI) = 0.6899. Values above branches are confidence levels estimated by 100 bootstrap replicates.

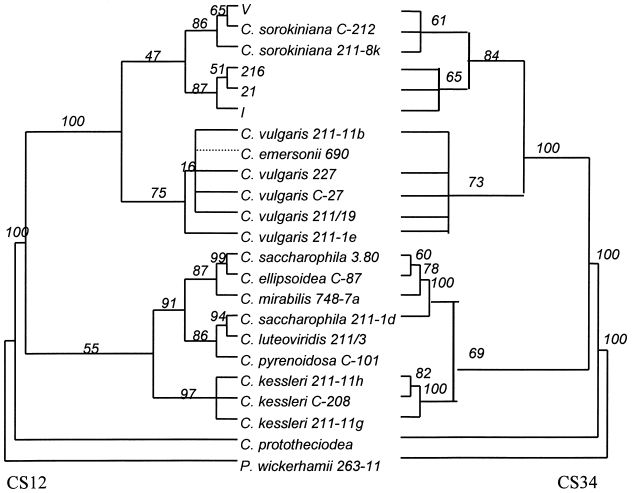

Figure 2. Phylogenetic relationships between CS12 and CS34 nucleotide sequences data.

lyze the isolates from the field samples. Parsimony analysis of the nuclear data set resulted in an efficient method for identification of strains. This analytical result illustrated the taxonomic congruence between the morphological characteristics (Figure 1). The confidence of the *Chlorella* spp. cluster was higher, up to 100%, and four strains were closely associated with the *C. sorokiniana, C. vulgaris,* and *C. lobophora* clusters. Furthermore, strains I, 21, and 216 were closer to *C. sorokiniana* Prag A14 while strain V was closer to *C. sorokiniana* C-212 and *C. sorokiniana* 211-8k.

Phylogenetic Analysis of Chloroplast Data

Parsimony analysis of the chloroplast SSU CS12, CS34 region has demonstrated sufficient congruence with morphological characters, as using *P. wickerhamii* as the outgroup, the four isolated strains were most closely related to *C. sorokiniana* (Figure 2). Furthermore, strains 21, 216 and I were closer to each other, in the middle degree, and strains 21 and 216 were most closely related to each other. Strain V was also more closely related to *C. sorokiniana* C-212 and *C. sorokiniana* 211-8k, from the phylogenetic analysis of nuclear data, so the chloroplast data were more robust across methods of phylogenetic reconstruction than the nuclear data (Figure 3).

Some Data from Applying Traditional Methods

Table 5 summarizes the results of microscopic observations of field isolates. The cells are spherical, cup-shaped chloroplasts with pyrenoids making it extremely difficult to differentiate them from one another. Under field conditions the algae propagated mostly via the two and four autospore formation.

At the upper temperature range, the growth experiment showed the four strains were all grown thermophilic, to 36°C. This finding also indicated that all isolates could be placed in the *C. sorokiniana* species with similar results in the molecular investigation. Starch hydrolysis testing revealed that strain V tested negative for starch hydrolysis ability, while strains 21, 216 and I all possessed proven starch hydrolysis ability coinciding with the results for *C. sorokiniana* Prag A14 (Table 5).

Discussion

Parsimony analyses in nuclear-encoded SSU rDNA, *Chlorella* spp. exhibited 90-100% similarity in the NS12 region. Meanwhile, *Chlorella* spp. cluster confidence

Table 5. Morphology characters, growth limited temperature and starch hydrolysis of 4 *Chlorella* spp.

Species	Cell form	Pyrenoid	Chloroplast form	Cell size (μm)	Temp. limit	Thermophily	Starch hydrolysis
C. sp. 21	Spherical	+	Cup-shaped	3~6	≒ 38°C	+	+
C. sp. 216	Spherical	+	Cup-shaped	3~6	≒ 38°C	+	+
C. sp. I	Spherical	+	Cup-shaped	3~6	≒ 38°C	+	+
C. sp. V	Spherical	+	Cup-shaped	3~6	≒ 41°C	+	-

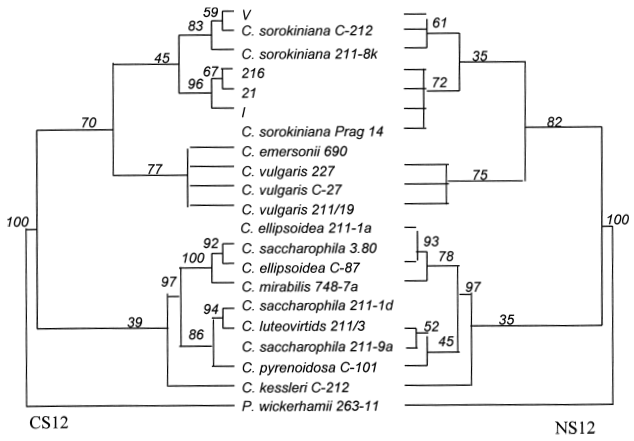

Figure 3. Phylogenetic relationships between NS12 and CS12 nucleotide sequences data.

reached 100%, indicating that *Chlorella* genes had a unique sequence in this region. This feature could be used to distinguish them from non-*Chlorella* species. Four isolates (strains 21, 216, I, V) were located on the *C. sorokiniana* lineage, and their similarity, up to 98%, was sufficient to consider them the same species. For phylogenetic analyses in this investigation, the morphological characteristics were compared and the same result was reached. Algae with cup-shaped chloroplast were clustered together, and other characteristics, such as the presence of pyrenoid, were also consistent with this finding (Figure 1). Phylogenetic analysis values on non-cup-shaped chloroplast algae were not high, and perhaps taxonomic characters here were more complicated. *Chlorella ellipsoidea* and *C. saccharophila* were ellipsoid-shaped algae. Although *C. ellipsoidea* and *C. mirabilis* shared a similar pyrenoid structure, *C. saccharophila* and *C. luteoviridis* shared a similar chloroplast structure. These characteristics made phylogenetic analyses more difficult.

Parsimony analyses of chloroplast-encoded SSU rDNA CS12 and CS34 regions was conducted herein. Apparently, these four isolates more closely resembled the species *C. sorokiniana*, rather than *C. vulgaris*. Chloroplasts with cup-shaped species are all clustered together. Strains 21, 216 and I were relatively close to one another, and strain V, *C. sorokiniana* C-212 and *C. sorokiniana* 211-8k were also relatively close to one another. Meanwhile, strains 21 and 216 were the most closely related species, and they had been isolated from the same environment. Comparing nuclear-SSU NS12 region data with chloroplast SSU CS12 region data revealed that outgroup to belong to *C. wickerhamii.* Phylogenetic analysis results share the same tendency, and in unknown species analysis, chloroplast SSU data had results clearer than the conventional method (Figure 3).

In primary structure analysis (Table 4), chloroplast data had more variable regions than nuclear data. This phenomenon could increase the difficulty of getting phylogenetic results. The changeable chloroplast gene may exist because its replication was less compact than the nuclear gene, a situation similar to that found in prokaryotes (Cedergren et al., 1988; Peer et al., 1990). Chloroplast and mitochondria are the places for processing energy change, and they produce more freeradicals, leading to genetic variation.

Nuclear data were more prominent in *Chlorella* gene levels than the chloroplast data. However, combining chloroplast CS12 with CS34 regions improves phylogenetic analysis in *Chlorella* genus levels (data not shown), making the analysis similar to that of the nuclear NS12 region. Perhaps the chloroplast gene was more variable among the species, making this region more suitable for species analysis. Combining the two regions into a longer sequence, enhances its phylogenetic analysis (Buchheim et al., 1996).

The molecular classification was examined using conventional methods. Microscopic observation of four isolates revealed significant similarities to one another, and also that they were congruent with the species *C. sorokiniana*. Elevated growth temperature also indicated a similar observation. According to Kessler (1982) and Huss et al. (1999), *C. sorokiniana* is the only species of *Chlorella* capable of growing above 36°C. Notably, strain V had the highest growth temperature. Kessler (1982) reported that *C. sorokiniana* 211-8k and *C. sorokiniana* Prag A14 exhibited differing starch hydrolysis ability. Therefore, in this study, we examined their biochemical characteristics. We suspect that strains 21, 216 and I possessed this ability as did *C. sorokiniana* Prag A14. Meanwhile, strain V did not perform the same as *C. sorokiniana* 211-8k. This molecular method of ribosomal DNA sequences was employed not only to reveal unknown strains, but also to reveal strain relationships through phylogenetic analysis. The process applied herein could be applied to classifying other unknown species of green algae.

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Literature Cited

Beijerinck, M.W. 1890. Culturversuche mit Zoochlorellen, Lichenen-goniden und anderen niederen Algen. Bot. Ztg. **45:** 726-739, 741-753, 757-767, 781-784.

Buchheim, M.A., C. Lemieux, C. Otis, R.R. Gutell, R.L. Chapman, and M. Turmel. 1996. Phylogeny of the Chlamydomonadales (Chlorophyceae): A comparison of ribosomal RNA gene sequences from the nucleus and the chloroplast. Mol. Phyl. Evol. **5:** 391-402.

Cedergren, R., M.W. Gray, and D. Sankoff. 1988. The evolutionary relationships among known life forms. J. Mol. Evol. **28:** 98-112.

Fott, B. and M. Novakova. 1969. A monograph of the genus *Chlorella*. *In* B. Fott (ed.), The Freshwater Species, Stud-

ies in Phycology. Academia, Praha, pp. 10-74.

Gilbert, D.G. 1992. SeqApp: A Biosequence Editor and Analysis Application. Version 1.9. Writen using MacApp, 1985-1991 Apple Computer, Inc.

Hoek, C., D.G. Mann, and H.M. Johns. 1995. Algae – An Introduction to Phycology. Cambridge University Press.

Hseu, R.S., H.H. Wang, H.F. Wang, and J.M. Moncalvo. 1996a. Differentiation and grouping of isolates of the *Ganoderma lucidum* complex by random amplified polymorphic DNA-PCR compared with grouping on the basis of internal transcribed spacer sequences. Appl. Environ. Microbiol. **62:** 1354-1363.

Hseu, R.S., J.M. Moncalvo, H.F. Wang, and H.H. Wang. 1996b. Application of PCR-amplified DNA to differentiate the *Ganoderma* isolates. J. Chinese Agri. Chem. Soc. **34:** 129-143.

Huss, V.A.R. and M.L. Sogin. 1990. Phylogenetic position of some *Chlorella* species within the Chlorococcales based upon complete small-subunit ribosomal RNA sequences. J. Mol. Evol. **31:** 432-442.

Huss, V.A.R., C. Frank, E.C. Hartmann, M. Hirmer, A. Kloboucek, B.M. Seidel, P. Wenzeler, and E. Kessler. 1999. Biochemical taxonomy and molecular phylogeny of the genus *Chlorella* sensu lato (Chlorophyta). J. Phycol. **35:** 587-598.

Kalina, T. and M. Puncocharova. 1987. Taxonomy of the subfamily Scotiellocystoideae Fott 1976 (Chlorellaceae, Chlorophyceae). Arch. Hydrobiol. Suppl. 73.4 (Algological Studies) **45:** 437-521.

Kessler, E. 1982. Chemotaxonomy in the Chlorococcales. *In* F. E. Round and D.J. Chapman (eds.), Progress in Phycological Research, Vol. 1. Elsevier, Amsterdam, pp. 111-135.

Kessler, E. 1984. A general view on the contribution of chemotaxonomy to the systematic of green algae. Systematics Association, Special vol. 17, Academic Press, London, pp. 391-407.

Komárek, J. and B. Fott. 1983. Das Phytoplankton des Suesswassers. Systematik und Biologie. 7. Teil, 1 Haelfte. Chlorophyceae (Gruenalgen). Ordnung Chlorococcales, *In* G. Huber-Pestalozii, (ed.), Die Binnengewasser 16. E. Schweizerbart'sche Verlagsbuchhandlung (Naegele & Obermiller), Stuttgart, pp. 1-1044.

Krienitz, L., V.A.R. Huss, and C. Hümmer. 1996. Picoplanktonic *Choricystis* species (Chlorococcales, Chlorophyta) and problems surrounding the morphologically similar '*Nannochloris*-like algae'. Phycologia **35:** 332-341.

Mullis, K.B., F.A. Faloona, S.J. Scharf, R.K. Saiki, G.T. Horn, and H.A. Erlich. 1986. Specific enzymatic amplification of DNA in vitro: the polymerase chain reaction. Cold Spring Harbor Symo. Quant. Biol. **51:** 263-273.

Mullis, K.B. and F.A. Faloona. 1987. Specific synthesis of DNA in vitro via a polymerase-catalyzed chain reaction. Methods Enzymol. **155:** 335-350.

Peer, Y.V., J.M. Neefs, and R. Wachter. 1990. Small ribosomal subunit RNA sequences, evolutionary relationships among different life forms, and mitochondrial origins. J. Mol. Evol. **30:** 463-476.

Saiki, R.K., D.H. Gelfand, and S. Toffel. 1988. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science **239:** 487-491.

Schreiner, M., M. Geisert, M. Oed, J. Arendes , U. Gungerich, H.J. Breter, K. Stuber, and D. Weinblum. 1995. Phylogenetic relationship of the green alga *Nanochlorum eucaryotum* deduced from its chloroplast rRNA sequences. J. Mol. Evol. **40:** 428-442.

Shihira, I. and R.W. Krauss. 1965. *Chlorella*. Physiology and Taxonomy of Forty-One Isolates. University of Maryland, College Park, Maryland, pp. 1-92.

Steinkötter, J., D. Bhattacharya, I. Semmelroth, C. Bibeau, and M. Melkonian.1994. Prasinophytes from independent lineages within the Chlorophyta: evidence from ribosomal RNA sequence comparisons. J. Phycol. **30:** 340-345.

Swofford, D.L. 1998. PAUP: Phylogenetic Analysis Using Parsimony. Version 4.01. Champaign: Illinois Natural History Survey.

Warburg, O. 1919. Uber die Geschwindigkeit der Kohlensàurezersetzung in lebenden Zellen. Biochem. Z. **100:** 20-270.

White, T.J., T. Bruns, S. Lee, and J. Taylor. 1990. Amplification and Direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR Protocols: A Guide to Methods and Applications, Copyright 1990 by Academic Press, Inc., pp. 315-322.

Wilcox, L.W., L.A. Lewis, P.A. Furest, and G.L. Floyd. 1992. Assessing the relationships of autosporic and zoosporic Chlorococcalean green algae with 18S rDNA sequence data. J. Phyol. **28:** 381-386.

利用核糖體核酸序列鑑定小球藻之分離株

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小球藻 *Chlorella* spp. 為目前被廣泛應用的微藻之一，由於 *Chlorella* spp. 為構造簡單之單細胞藻類，作為種鑑定依據的形態特徵常因生態環境改變而異，造成鑑定上的困難。為瞭解 *Chlorella* spp. 種間的親緣關係，本研究以分子生物技術中的聚合　連鎖反應放大特定區域，分析四株分別從台灣和印尼所分離之小球藻細胞核，及葉綠體內的核糖體之小次體基因序列，與基因資料庫已發表的基因序列加以比對，以進行小球藻屬藻株 *Chlorella* spp. 的分類鑑定，並與保存中心藻種，比較分類結果互相驗證。結果顯示供試的四株小球藻均接近於常見的綠藻 *Chlorella sorokiniana*，此為一可生長於 35°C 水域中的耐高溫小球藻，分析結果更顯示藻株 216 更接近於藻株 21 和 I。葉綠體 SSU rDNA 基因序列比細胞核者更清楚的分析出四株小球藻的親緣關係。藻株 21 與 216 跟藻株 I 較為接近。從形態與生化性質比較驗證後，顯示此 rDNA分類結果與傳統分類方法所得結果，呈強烈的相關性。

關鍵詞：小球藻；核糖體之小次體；細胞核；葉綠體；聚合酶連鎖反應；系統發生學。