Revista de Biología Marina y Oceanografía
Vol. 49, Nº3: 615-623, diciembre 2014
DOI 10.4067/S0718-19572014000300019

RESEARCH NOTE

Molecular and phylogenetic identification of an oil-producing strain of *Nannochloropsis oceanica* (Eustigmatophyceae) isolated from the southwestern Atlantic coast (Argentina)

Identificación molecular y filogenética de una cepa oleaginosa de *Nannochloropsis oceanica* (Eustigmatophyceae) aislada de la costa Atlántica suroeste (Argentina)

Natalia Bongiovani[1], M. Virginia Sanchez-Puerta[2], Cecilia Popovich[1,3] and Patricia Leonardi[1,3]

[1]Laboratorio de Estudios Básicos y Biotecnológicos en Algas y Hongos (LEBBAH), Centro de Recursos Naturales Renovables de la Zona Semiárida (CERZOS) –CONICET, Camino La Carrindanga, Km 7, 8000, Bahía Blanca, Argentina
[2]IBAM-CONICET, Instituto de Ciencias Básicas, Facultad de Ciencias Agrarias, Universidad Nacional de Cuyo, Alte. Brown 500, 5505, Chacras de Coria, Mendoza, Argentina. mvsanchezpuerta@fca.uncu.edu.ar
[3]Laboratorio de Ficología y Micología, Departamento de Biología, Bioquímica y Farmacia, Universidad Nacional del Sur, San Juan 670, 8000, Bahía Blanca, Argentina

Abstract.- Screening of local microalgae species with potential for oil production is essential to achieve successful commercial large-scale cultures. In this study, identification of a South American species of *Nannochloropsis* was carried out using molecular and phylogenetic analyses of chloroplastic and nuclear genes, *rcbL* and 18S rDNA, respectively. The gene sequences for the studied strain were highly similar to other strains of *Nannochloropsis oceanica* (100% for 18S rDNA and 99.7% for *rbcL*) isolated from the Red Sea or Mediterranean Sea (Israel) and from the Pacific Ocean (Japan).

Key words: *Nannochloropsis oceanica*, *rcbL*, 18S rDNA, oil

INTRODUCTION

The decrease in fossil fuel reserves, as well as a sharp increase in oil prices, has intensified the search for alternative renewable energy sources. This concern has promoted a special interest in developing third-generation biofuels, which are produced from renewable feedstock, such as algal biomass, and in particular biodiesel from microalgal lipids (Pruvost 2011). For sustainable production of algal-derived biodiesel, exploitation of local microalgae is advantageous. However, the appropriate selection of fast-growing, lipid-producing microalgae strains that are adapted to local climatic conditions constitutes one of the major challenges faced by researchers worldwide. The first step in these studies includes species selection, which is essential for a reliable analysis. In turn, this requires recognizing the diagnostic characteristics of different microalgal groups to achieve a correct identification (Leonardi *et al.* 2011). Traditionally, light microscopy and transmission electron microscopy were standard procedures for identification and characterization of microalgae species. Nevertheless, these methods were not sufficient for accurate species identification in several algal lineages (Karlson *et al.* 1996).

The genus *Nannochloropsis* comprises 6 species: *N. oculata* (Droop) Hibberd (Hibberd 1981), *N. salina* Hibberd (Hibberd 1981), *N. gaditana* Lubian (Lubian 1982), *N. granulata* Karlson & Potter (Karlson *et al.* 1996), *N. limnetica* (Krienitz *et al.* 2000) and *N. oceanica* (Suda *et al.* 2002). Taxonomic identification of *Nannochloropsis* species is challenging due to the small cell size and simple structure (Maruyama *et al.* 1986, Gladu *et al.* 1995), the difficulties in fixing the cells for transmission electron microscopy (Hibberd 1981) and the lack of sexual reproduction. Even though meiosis-related genes were found in the genome of *N. gaditana*, no transcripts were detected (Radakovits *et al.* 2012).

Some species of *Nannochloropsis*, such as *Nannochloropsis* sp. (Sukenik *et al.* 1989), *N. oculata* (Renaud *et al.* 1991, Li *et al.* 2009) and *N. gaditana* (Ferreira *et al.* 2009) are used in marine aquaculture as an important source of eicosapentaenoic acid. Moreover, different species of *Nannochloropsis* have recently been considered appealing feedstock for biodiesel production due to their ability to accumulate high amounts of lipids.

For example, *Nannochloropsis* sp. (Rodolfi *et al.* 2009, Pal *et al.* 2011, Bondioli *et al.* 2012), *N. gaditana* (Simionato *et al.* 2011, 2013), *N. oculata* (Van Vooren *et al.* 2012), *N. salina* (Sforza *et al.* 2012) and *N. oceanica* (Dong *et al.* 2013, Pal *et al.* 2013, Bongiovani *et al.* 2013, Solovchenko *et al.* 2014). Given the importance of *Nannochloropsis* as an oleaginous species for biodiesel production, a number of genomic and transcriptomic studies on several species of the genus have been recently published (Radakovits *et al.* 2012, Vieler *et al.* 2012, Wei *et al.* 2013, Starkenburg *et al.* 2014, Wang *et al.* 2014, Carpinelli *et al.* 2013, Hu *et al.* 2014, Li *et al.* 2014).

Because the ability to produce large quantities of lipids is species-specific (Hu *et al.* 2008), a correct specific identification is critical. Thus, the phylogenetic species concept becomes particularly useful (Andersen *et al.* 1998). Species of *Nannochloropsis* have been delimited by DNA sequence analysis (Fawley & Fawley 2007) based on nuclear (18S rDNA) and chloroplastic (*rbcL*) markers and sets of orthogonal genes (Krienitz *et a*l. 1996, Andersen *et al.* 1998, Suda *et al.* 2002, Vieler *et al.* 2012, Cao *et al.* 2013, Wang *et al.* 2014).

In this study, we identified a new strain of *Nannochloropsis oceanica* CCALA 978 based on molecular taxonomy and studied its evolutionary relationships based on phylogenetic analyses with nuclear and chloroplastic genes. Recent studies have demonstrated the potential suitability of this strain isolated from the southwestern Atlantic coast for biodiesel production (Bongiovani *et al.* 2013; Bongoviani *et al.* 2013[1]).

Materials and Methods

Strain and culture conditions

Nannochloropsis oceanica (CCALA 978, Culture Collection of Autotrophic Organism, Institute of Botany, Academy of Sciences of the Czech Republic) was isolated from the southwestern Atlantic coast (65°01'W, 43°18'S, Argentina) and kindly provided by CRIAR, Instituto de Biología Marina y Pesquera Almirante Storni, San Antonio Oeste, Río Negro province, Argentina. This species was cultured in f/2 marine medium (Guillard 1973). Cultures were maintained in flasks under the following environmental conditions: 16°C, 60 μE m^{-2} s^{-1} and 12:12 light:dark period. Cultures were continuously bubbled with air; 1-2% CO_2 was mixed in the air stream and this mixture was applied during 3-4 h per day.

Molecular analysis

DNA extraction and sequencing

Approximately 1L of culture was harvested by centrifugation, flash-frozen in liquid nitrogen and stored at -80°C. Genomic DNA was extracted using Illustra Nucleon Phytopure Genomic DNA extraction kit (GE Healthecare, Buckinghamshire, UK) and kept at -20°C until analysis.

The nuclear-encoded 18S small subunit (SSU) rDNA and the chloroplast-encoded *rbcL* genes were amplified using the following published primers: for *rbcL*, rbcLF: 5'GATGCAAACTACACAATTAAAGATACTG3' and rbcLR: 5'ATTTTGTTCGTTTGTTAAATCCG3' (Li *et al.* 2011); and for 18S rDNA, 18SrDNAF: 5'CAAGTTTC TGCCCTATCAGCT3' and 18S rDNAR: 5'GCTTTCG CAGTAGTTCGTCTT3' (Li *et al.* 2011), NS3a: 5'GCAAGT CTGGTGCCAGCAGCC3' (Fawley *et al.* 2005), Primer A: 5'CCGAATTCGTCGACAACCTGGTTGATCCTGCCAGT3' (Medlin *et al.* 1988) and Primer B: 5'CCCGGGATCCA AGCTTGATCCTTCTGCAGGTTCACCTAC3' (Medlin *et al.* 1988). Amplification products were sequenced by Sanger sequencing with Applied Biosystems 3730XL (Life Technologies) and sequences were deposited in GenBank (accession numbers KF010153 and KF010154 for *rbcL* and 18S rDNA, respectively). Sequences were aligned using MEGA 5.1 (Tamura *et al.* 2011). GenBank accession numbers and taxonomic data of *Nannochloropsis* species included in the *rbcL* and 18S rDNA alignments are listed in Table 1.

Phylogenetic analyses

Phylogenetic analyses were performed separately for each data set. Maximum Parsimony (MP) analyses were done with PAUP*4b10 (Swofford *et al.* 2002). For MP analyses, characters were unweighted and a heuristic search was used with the tree bisection and reconnection (TBR) branch-swapping method, and addition was random with 10 repetitions. JModeltest (Posada & Crandall 1998) was used to select the best model of DNA substitution for the Maximum Likelihood (ML) analyses according to the Akaike information criterion (AIC). For both genes, the GTR+I+G4 model was selected. The Maximum Likelihood analyses were done with Garli 0.951 (Zwickl 2006) under the General Time Reversible model with parameters for invariable sites and gamma-distributed rate heterogeneity. As outgroups, *Pseudotraedriella* (EF044311) and *Eustigmatus magnus* (AB280615), and *Vischeria helvetica* (HQ710612) were used for 18S rDNA and *rbc*L analyses, respectively. A hundred bootstrap replicates were done for the ML and MP analyses.

[1]Bongiovani N, AM Martínez, C Popovich, D Constenla & PI Leonardi. 2013. Efecto de la deficiencia de nitrógeno y la intensidad de luz sobre la productividad y composición lipídica en *Nannochloropsis oceanica*. HYFUSEN. 5° Congreso Nacional- 4° Congreso Iberoamericano. Hidrógeno y Fuentes Sustentables de Energía. p. 198.

Table 1. Taxonomic data, collection site and GenBank accession number of taxa in the *rbcL* or 18S rDNA alignment / Datos taxonómicos, sitios de recolección y número de código de GenBank de los taxa de los alineamientos de *rbcL* y 18S rDNA

Species	Strain	Collection Site	GenBank Accession Number	
			rbcL	18S rDNA
N. oceanica	CCALA 978	Southwestern Ocean Atlantic Cost	KF010153 (this study)	KF010154 (this study)
N. oculata	CCAP 849	Skate Point,Isle of Cumbrae, Scotland, UK	AB052286	-
N. oculata	CCMP525	Skate Point, Isle of Cumbrae, Scotland, UK	HQ710609	AF045044
N. oculata	CCMP533	Lake of Tunis, Tunesia, North Africa	-	AY045045
N. granulata	MBIC10054	Pacific Ocean, 32°12' N, 147°23' E	AB052280	AB052272
N. granulata	BDH02	Unknown	KC 128502	KC128500
N. granulata	CCMP1662	Skagerrak, North Sea	-	AF045041
N. granulata		Skagerrak, North Sea		NGU38903
N. limnetica	KR1998	Arrowodd National Wildlife Refuge, Itasca Lake Park, Minessota, USA	DQ977729	-
N. limnetica	JL1125	Arrowodd National Wildlife Refuge, Itasca Lake Park, Minessota, USA	DQ977730	-
N. limnetica	DML1114	Arrowodd National Wildlife Refuge, Itasca Lake Park, Minessota, USA	DQ977740	-
N. limnetica	AS39	Arrowodd National Wildlife Refuge, Itasca Lake Park, Minessota, USA	DQ977741	DQ977726
N. limnetica	AS2168	Arrowodd National Wildlife Refuge, Itasca Lake Park, Minessota, USA	DQ977739	-
N. limnetica	SAG1899	Lake Roter See, Mecklenburg-Vorpommern, Germany	AM421006	AF251496
N. limnetica	JL24	Arrowodd National Wildlife Refuge, Itasca Lake Park, Minessota, USA	-	DQ977727
N. gaditana	MBIC10118	Shell Beach, Australia	AB052279	AB052269
N. gaditana	MBIC10123	Monkey Mia, Australia	AB05273	AB052271
N. gaditana	MBIC10418	Atlantic Ocean, Cape Town, South Africa	AB052735	AB052271
N. gaditana	Ferrara & Andreoli 2004	Comachio Lagoon, Ferrara, Italy	-	AF133819
N. gaditana	IVP	Unknown	-	AB473733
N. gaditana	CCAP849/5-CCMP1775	Cadiz Bay, Cadiz, Spain	-	AF067957
N. gaditana	CCMP526	Lagune di Quadilia, Morocco	-	AF045037
N. gaditana	B	Unknown	-	JF444989
N. gaditana	CCAP849/5-CCMP1775	Cadiz Bay, Cadiz, Spain	-	AF045036
N. salina	CCAP849/2	Skate Point, Isle of Cumbrae, Scotland, UK	AB052288	AF045046
N. salina	MBIC10063	Pacific Ocean, 41°28'N, 146°57'E	AB052287	AB052278
N. oceanica	MBIC10179	Red Sea, Eilat, Israel	AB052283	AB052275
N. oceanica	MBIC10176	Mediterranean Sea, Haifa, Israel	AB052272	AB052274
N. oceanica	MBIC10426	Red Sea, Eilat, Israel	AB052284	AB052276
N. oceanica	MBIC10440	Red Sea, Eilat, Israel	AB052285	AB052277
N. oceanica	MBIC10090	Pacific Ocean, Off Sanriku, Japan	AB052281	AB052273
N. oceanica	LAMB001	Unknown	HQ201773	-
N. oceanica	EUS001	Unknown	-	HQ710567
N. oceanica	CCAP211/46	Kuwait	-	AF045034
N. oceanica	CCAP211/78	Unknown	-	AF045035
Nannochloropsis sp.1	CCMP531	Qingdao, China	-	U41094
Nannochloropsis sp.2	CCMP505	Morehead City, USA	-	U41050
Nannochloropsis sp.	UTEX2379	Unknown	-	AY560119

Results and discussion

Cells were spherical, with diameters of 2-3 μm and 3-5 μm under exponential and stationary growth phases, respectively. Light microscopic observations showed a smooth cell wall, a parietal chloroplast and an eyespot that was always present (Fig. 1). Cell dimensions of the isolate under study agree with the dimensions indicated for other strains of *N. oceanica* (Suda *et al.* 2002, Cao *et al.* 2013). However, the eyespot, which was constantly present in the algal strain under study and also in some cells of *N. oceanica* var. *sinensis* (Cao *et al.* 2013), was rarely observed by Suda *et al*. (2002). Our previous ultrastructural study showed that the cells of *N. oceanica* CCALA978 had a nucleus, a single parietal chloroplast and a thick cell wall (Bongiovani *et al.* 2013a). In this strain, the cell wall papilla and the pyrenoid-like structure described in *N. oceanica* and other species of *Nannochloropsis* by Suda *et al*. (2002) and Cao *et al.* (2013) were not observed. These authors did not find morphological traits to distinguish among *N. granulata*, *N. salina*, *N. gaditana* and *N. oceanica* by means of light microscopy or transmission electron microscopy.

Sequences for 18S rDNA and *rbcL* genes were used to identify several microalgal species and in particular to differentiate species of *Nannochloropsis* (Karlson *et al*. 1996, Krienitz *et al*. 2000). Here, the molecular studies based on 2 genes allowed us to identify the isolated strain CCALA978 as *N. oceanica*. The gene sequences for the strain were highly similar (100% for 18S rDNA and 99.7% for *rbcL*) to other strains of *N. oceanica* isolated from different marine habitats (Table 1). The intraspecific variation for the gene *rbcL* within the species *N. oceanica* (100% for 18S rDNA and 99.7-100% for *rbcL*) was comparable to that of other species of the genus (99.8-99.9% identity within *N. limnetica* and 98.6-100% within *N. gaditana*). The phylogenetic analyses based on the *rbcL* (Fig. 2) and 18S rDNA (Fig. 3) genes resulted in a similar topology separating 2 main clades: *N. oculata+N. oceanica+N. limnetica+N. granulata* and *N. gaditana+N. salina* with high bootstrap support values. This result was also observed by Andersen *et al.* (1998), Krienitz *et al.* (1996) and Vieler *et al.* (2012) based on 18S rDNA; by Suda *et al*. (2002) and Cao *et al.* (2013) based on 18S rDNA and *rbcL*; and by Wang *et al.* (2014) based on 1085 single-copy nuclear orthologous gene sets. This topology is consistent with a morphological difference between the 2 main clades. Species within the group of *N. gaditana+N. salina* are cylindrical in shape, while cells

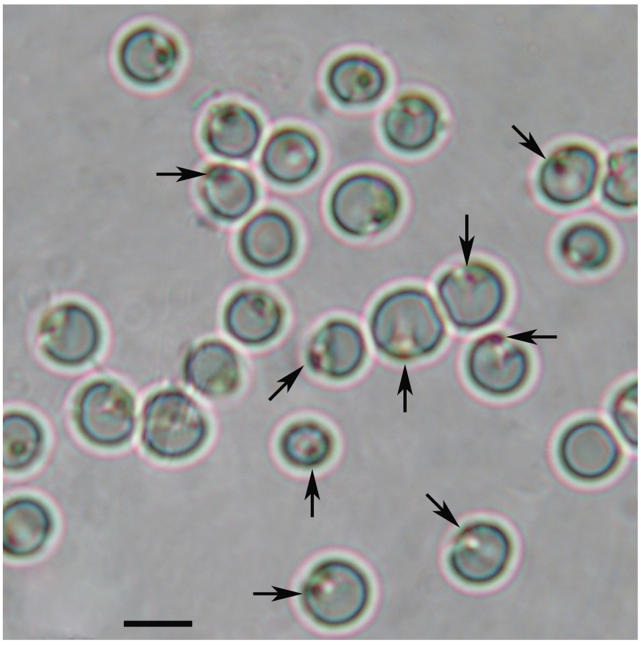

Figure 1. Light micrograph of *Nannochloropsis oceanica*. Arrows indicate the eyespot. Scale bar= 3 μm / Micrografía óptica de *Nannochloropsis oceanica*. Las flechas indican el estigma. Barra de escala= 3 μm

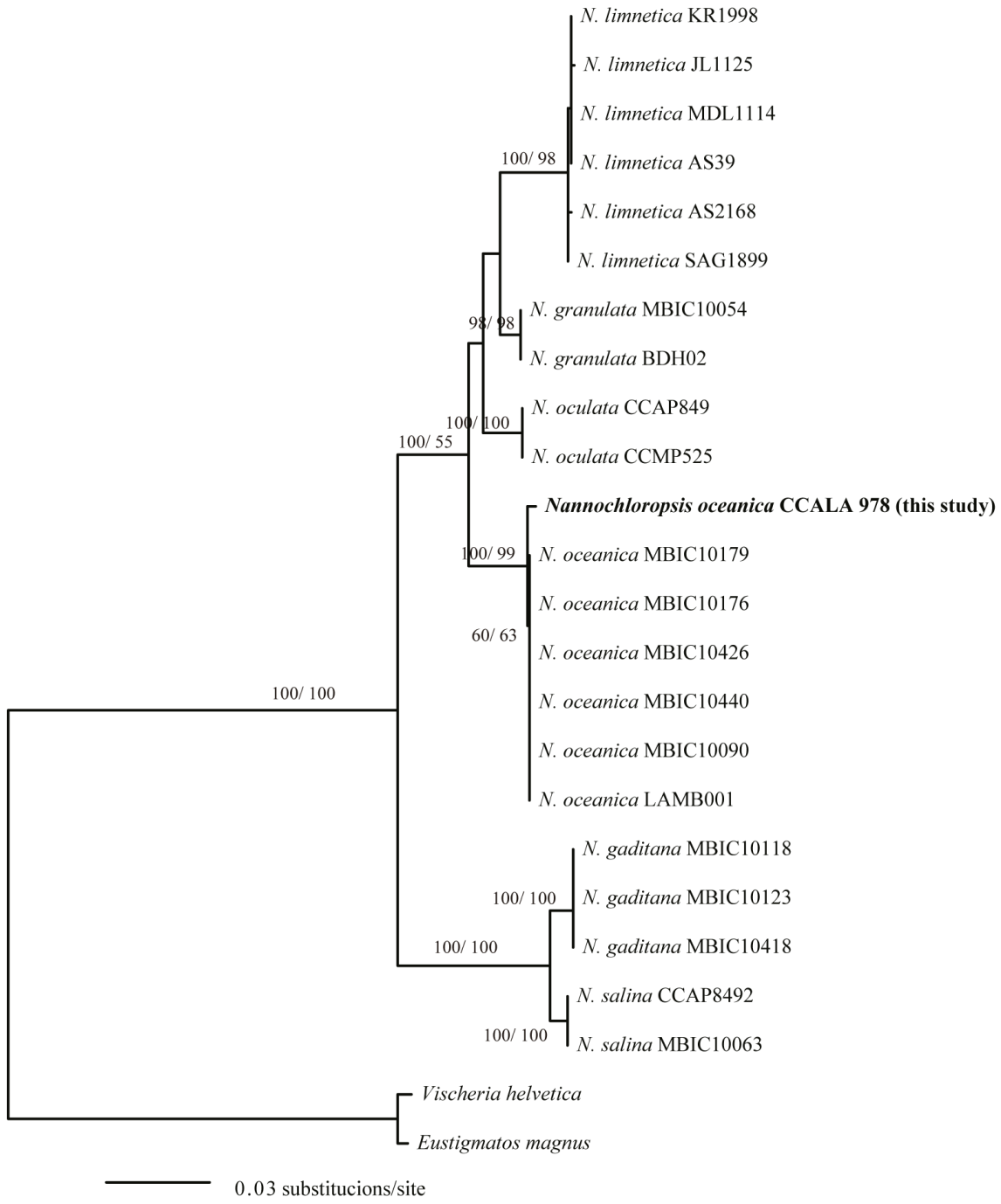

Figure 2. Maximum Likelihood (ML) phylogenetic tree based on the chloroplast-encoded *rbcL* (1389 nt) using Garli under the GTR+I+G4 model selected by JModelTest. Maximum Parsimony (MP) analyses were done with PAUP*4b10. Bootstrap values (100 replicates) from MP (left) and ML analysis (right) are provided when >50% / Árbol filogenético bajo el criterio de Máxima Verosimilitud (ML) basado en el gen cloroplastídico *rbcL* (1389 nt) usando Garli con el modelo GTR+I+G4 seleccionado por JModelTest. Los análisis bajo el criterio de Máxima Parsimonia (MP) fueron realizados con PAUP*4b10. Los valores de soporte estadístico (100 réplicas) bajo MP (izquierda) y bajo ML (derecha) se encuentran indicados si >50%

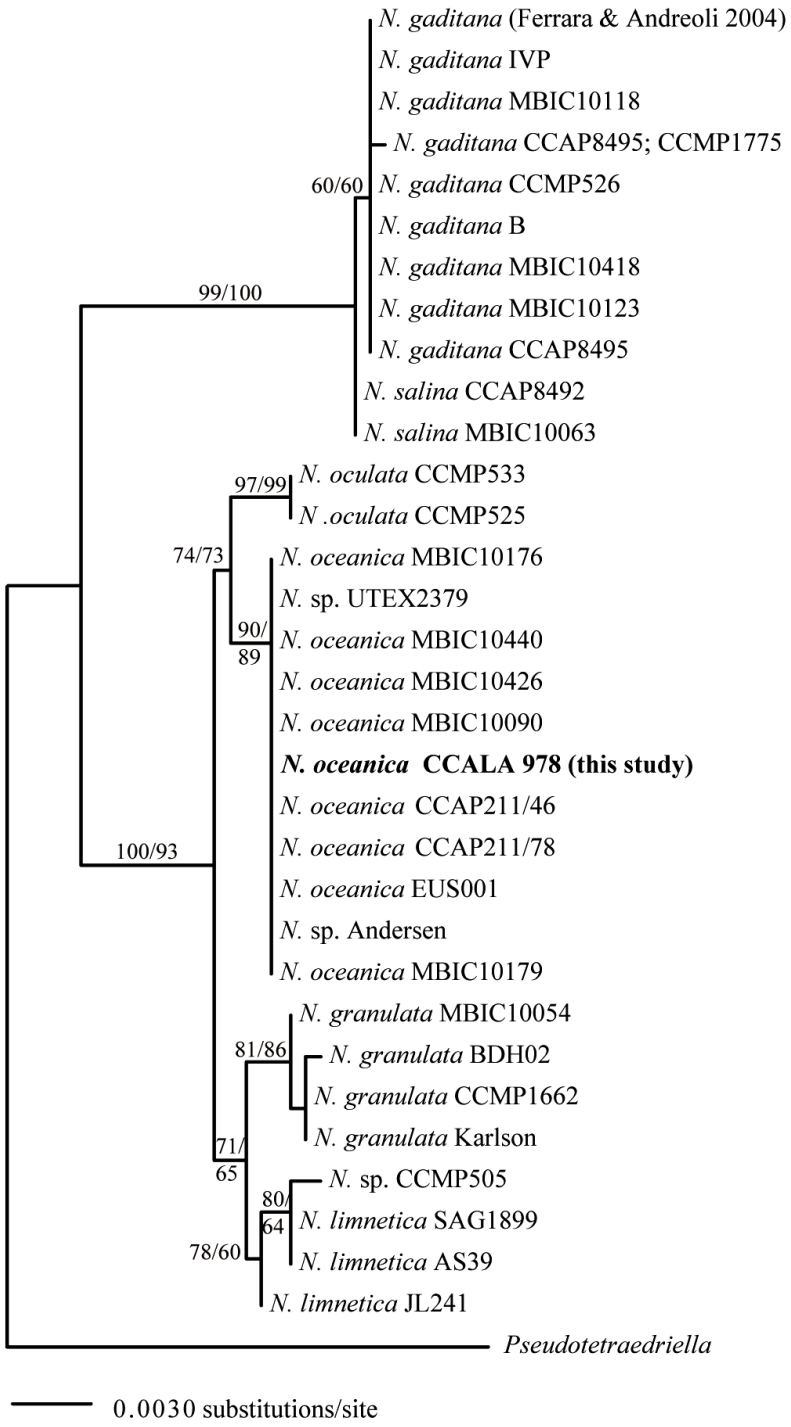

Figure 3. Maximum Likelihood phylogenetic tree based on the nuclear gene 18S rDNA (1792 nt) using Garli under the GTR+I+G4 model selected by JModelTest. Maximum Parsimony (MP) analyses were done with PAUP*4b10. Bootstrap values (100 replicates) from MP (left) and ML analysis (right) are provided when >50% / Árbol filogenético bajo el criterio de Máxima Verosimilitud (ML) basado en el gen nuclear rDNA (1792 nt) usando Garli con el modelo GTR+I+G4 seleccionado por JModelTest. Los análisis bajo el criterio de Máxima Parsimonia (MP) fueron realizados con PAUP*4b10. Los valores de soporte estadístico (100 réplicas) bajo MP (izquierda) y bajo ML (derecha) se encuentran indicados si >50%

in species of the other clade are spherical to oval (Hibberd 1981, Lubian 1982, Karlson *et al*. 1996).

Microalgae exhibit environmental-tolerance ranges that are species-specific. Thus, a correct identification at species level ensures appropriate conditions to achieve a profitable and successful culture at large scale. In this study, an appropriate identification of a South American oleaginous microalgal strain of *Nannochloropsis oceanica* (CCALA 978) contributes to standardize processes towards biodiesel production.

Acknowledgments

This study was supported by grants from Consejo Nacional de Investigaciones Científicas y Técnicas de la República Argentina (CONICET), PIP 112-201101-00208, Agencia Nacional de Promoción Científica y Tecnológica, PICT 2010-0959 and PICT 2008-277 and Secretaría de Ciencia y Tecnología de la Universidad Nacional del Sur, PGI TIR. MVSP and PIL are researchers of the CONICET, Argentina.

Literature cited

Andersen R, R Brett, D Potter & J Sexton. 1998. Phylogeny of the Eustigmatophyceae based upon 18S rDNA, with emphasis on *Nannochloropsis*. Protist 149: 61-74.

Bondioli P, L Della-Bella, G Rivolta, G Chini-Zittelli, N Bassi, L Rodolfi, D Casini, M Prussi, D Chiaramonti & MR Tredici. 2012. Oil production by the marine microalgae *Nannochloropsis* sp. F&M-M24 and *Tetraselmis suecica* F&M-M33. Bioresource Technology 114: 567-672.

Bongiovani N, C Popovich, AM Martínez, H Freije, D Constenla & PI Leonardi. 2013. *In vivo* measurements to estimate culture status and neutral lipid accumulation in *Nannochloropsis oculata* CCALA 978: implications for biodiesel oil studies. Algological Studies 142: 3-16.

Cao S, X Zhang, X Fan, H Qiao, C Liang, D Xu, S Mou, W Wang & N Ye. 2013. Phylogeny and characterization of *Nannochloropsis oceanica* var. *sinensis* var. nov. (Eustigmatophyceae), a new oleaginous alga from China. Phycologia 52: 573-577.

Carpinelli EC, A Telatin, N Vitulo, C Forcato, M D'Angelo, R Schiavon, A Vezzi, GM Giacometti, T Morosinotto & G Valle. 2013. Chromosome scale genome assembly and transcriptome profiling of *Nannochloropsis gaditana* in nitrogen depletion. Molecular Plant 6: 323-335.

Dong HP, E Williams, D Whang, X Zhang, R Hsia, A Jenck, R Halden, J Li, F Cheng & AR Place. 2013. Responses of *Nannochloropsis oceanica* IMET1 to long-term nitrogen starvation and recovery. Plant Physiology 162: 1110-1126.

Fawley KP & MW Fawley. 2007. Observations on the diversity and ecology of freshwater *Nannochloropsis* (Eustigmatophyceae) with descriptions of new taxa. Protist 158: 325-336.

Fawley M, K Fawley & H Owen. 2005. Diversity and ecology of small coccoid green algae from Lake Itasca, Minnesota, USA, including *Meyerella planktonica*, gen. et sp. nov. Phycologia 44: 35-48.

Ferreira M, P Coutinho, P Seixas, J Fábregas & A Otero. 2009. Enriching rotifers with 'premium' microalgae *Nannochloropsis gaditana*. Marine Biotechnology 11: 585-595.

Gladu PK, G Patterson, GH Wikfors & BC Smith. 1995. Sterol, fatty acid and pigment characteristics of UTEX 2341, a marine Eustigmatophyte identified previously as *Chlorella minutissima* (Chlorophyceae). Journal of Phycology 31: 774-777.

Guillard RRL. 1973. Division rates. In: Stein JR (ed). Handbook of phycological methods: Culture methods and growth measurements, pp 289-311. Cambridge University Press, Cambridge.

Hibberd DJ. 1981. Notes on the taxonomy and nomenclature of the algal classes Eustigmatophyceae and Tribophyceae (synonym Xanthophyceae). Botanical Journal of the Linnean Society 82: 93-119.

Hu J, D Wang, J Li, G Jing, K Ning & J Xu. 2014. Genome-wide identification of transcription factors and transcription-factor binding sites in oleaginous microalgae *Nannochloropsis*. Nature, Scientific Report 4: 5454-5464.

Hu Q, M Sommerfeld, E Jarvis, M Ghirardi, M Posewitz M Seibert & A Darzins. 2008. Microalgal triacylglycerols as feedstocks for biofuel production: perspectives and advances. Plant Journal 54: 621-639.

Karlson B, D Potter, M Kuylenstierna & R Andersen. 1996. Ultrastructure, pigment composition, and 18S rRNA gene sequence for *Nannochloropsis granulata* sp. nov. (Monodopsidaceae, Eustigmatophyceae), a marine ultraplankter isolated from the Skagerrak, northeast Atlantic Ocean. Phycologia 35: 253-260.

Krienitz L, D Hepperle, HB Stich & W Weiler. 2000. *Nannochloropsis limnetica* (Eustigmatophyceae), a new species of picoplankton from freshwater. Phycologia 39: 219-227.

Leonardi PI, C Popovich & C Damiani. 2011. Feedstock for second- generation biodiesel: microalgae's biology and oil composition. In: Dos Santos-Bernardes MA (ed). Economic effects of biofuel production, pp. 318-346. InTech Publisher, Rijeka.

Li F, D Gao & H Hu. 2014. High-efficiency nuclear transformation of the oleaginous marine *Nannochloropsis* species using PCR product. Bioscience, Biotechnology and Biochemestry 78: 812-817.

Li J, D Han, D Wang, K King, J Jia, L Wei, X Jing, S Huang, J Chen, Y Li, Q Hu & J Xu. 2014. Choreography of transcriptomes and lipidomes of *Nannochloropsis* reveals the mechanisms of oil synthesis in microalgae. The Plant Cell 26: 1645-1665.

Li S, K Pan, B Zhu, X Ma, X Liangm & G Yang. 2011. Molecular identification of a species in genus *Nannochloropsis*. Journal of Ocean University of China 10: 391-396.

Lubian L. 1982. *Nannochloropsis gaditana* sp. nov., una nueva Eustigmatophyceae marina. Lazaroa 4: 287-293.

Maruyama L, T Nakamura, T Matsubayashi, Y Ando & T Maeda. 1986. Identification of the algae known as 'marine *Chlorella*' as a member of the Eustigmatophyceae. Journal of Phycology 34: 319-325.

Medlin L, HJ Elwood, S Stickeland & ML Sogin. 1988. The characterization of enzimatically amplified eukaryotic 16S-like r RNA-coding regions. Gene 71: 491-499.

Pal D, I Khozin-Goldberd, Z Cohen & S Boussiba. 2011. The effect of light, salinity and nitrogen availability on lipid production by *Nannochloropsis* sp. Applied Microbiology and Biotechnology 90: 1429-1441.

Pal D, I Khozin-Goldberg, S Didi-Cohen, A Solovchenko, A Batushanky, Y Kaye, N Sikron, T Samani, A Faut & S Boussiba. 2013. Growth, lipid production and metabolic adjustments in the euryhalline eustigmatophyte *Nannochloropsis oceanica* CCALA 804 in response to osmotic downshift. Applied Microbiology Biotechnology 97: 8291-8306.

Posada D & K Crandall. 1998. ModelTest: testing the model of DNA substitution. Bioinformatics 14: 817-818.

Pruvost J. 2011. Cultivation of algae in photobioreactors for biodiesel production. In: Pandey A, C Larroche, SC Picke, CG Dussap & E Gnansounou (eds). Biofuels: Alternative feedstocks and conversion processes, pp. 439-461. Elsevier, Amsterdam.

Radakovits R, R Jinkerson, S Fuertenberg, H Tae, R Settlage, L Boore & M Posewitz. 2012. Draft genome sequence and genetic transformation of the oleaginous alga *Nannochloropsis gaditana*. Nature Communication 3: 686.

Renaud S, D Parry, L Thinh, C Kuo, A Padovan & N Sammy. 1991. Effect of light intensity on the proximate biochemical and fatty acid composition of *Isochrysis* sp., and *Nannochloropsis oculata* for use in tropical aquaculture. Journal of Applied Phycology 3: 347-356.

Rodolfi L, G Chini-Zittelli, N Bassi, G Padovani, N Biondi, G Bonini & M Tredici. 2009. Microalgae for oil: strain selection, induction of lipid synthesis and outdoor mass cultivation in a low cost photobioreactor. Biotechnology and Bioengineering 102: 100-112.

Sforza E, A Bertucco, T Morosinotto & G Giacometti. 2012. Photobioreactor for microalga growth and oil production with *Nannochloropsis salina*: from lab-scale experiments to large-scale design. Chemical Engineering Research and Design 90: 1151-1158.

Simionato D, E Sforza, E Corteggiani, A Carpinelli-Bertucco, GM Giacometti & T Morosinotto. 2011. Acclimation of *Nannochloropsis gaditana* to different illumination regimes: Effects on lipids accumulation. Bioresource Technology 102: 6026-6032.

Simionato D, MA Block, N La Rocca, J Jouhet, E Meréchal, G Finazzi & T Morosinotto. 2013. The response of *Nannochloropsis gaditana* to nitrogen starvation includes de novo biosynthesis of triacylglicerols, a decrease of chloroplast galactolipids, and reorganization of the photosynthetic apparatus. Eukaryot Cell 12: 665-676.

Solovchenko A, A Lukyanov, O Solovchenko, S Dihi-Cohen, S Boussiba & I Khozin-Goldberg. 2014. Interactive effects of salinity, high light, and nitrogen starvation of fatty acid and carotenoids profiles in *Nannochloropsis oceanica* CCALA 804. European Journal of Lipid Science Technology 116: 635-644.

Starkenburg SR, KJ Kwon, RK Jha, C McKay, M Jacpbs, O Chertkov, S Twary, G Rocap & RA Cattolico. 2014. A pangenomic analysis of the *Nannochloropsis* organellar genomes reveals novel genetic variation in key metabolic genes. BMC Genomic 15: 212- 233.

Suda S, M Atsumi & H Miyashita. 2002. Taxonomic characterization of a marine *Nannochloropsis* species, *N. oceanica* sp. nov. (Eustigmatophyceae). Phycologia 41: 273-279.

Sukenik A, Y Carmeli & T Berner. 1989. Regulation of fatty acid composition by irradiance level in the Eustigmatophyte *Nannochloropsis* sp. Journal of Phycology 25: 686-692.

Swofford D, G Olsen, P Waddell & D Hillis. 2002. PAUP*. Phylogenetic analysis using parsimony (and other methods).Version 4. Sinauer Associates, Sunderland.

Tamura K, D Peterson, N Peterson, G Stecher, M Nei & S Kumar. 2011. MEGA 5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Molecular Biology and Evolution 28: 2731-2739.

Van Vooren G, F Le Grand, J Legrand, S Cuiné, G Peltier & J Pruvost. 2012. Investigation of fatty acids accumulation in *Nannochloropsis oculata* for biodiesel application. Bioresource Technology 124: 421-432.

Vieler A, G Wu, C-H Tsai, B Bullard, AJ Cornich, C Harvey, I-B Reca, C Thornburg, R Achawanantakun, CJ Buehl, MS Campbell, D Cavalier, KL Childs, TJ Clarck, R Deshpande, E Erickson, AA Ferguson, W Handee, Q Kong, X Li, B Lui, S Lundback, C Peng, RL Roston, Sanjaya, JP Simpson, A Terbuch, J Warakanont, S Zäuner, EM Farre, EL Hegg, N Jiang, M-H Kuo, Y Lu, KK Niyogi, J Ohlrogge, KW Osteryoung, Y Shachar-Hill, BB Sear, Y Sun, H Takahashi, M Yandell, S-H Shiu & C Benning. 2012. Genome, functional and gen annotation and nuclear transformation of the heterokont oleaginous alga *Nannochloropsis oceanica* CCMP1779. PLOS Genetics 8: 1-25.

Wang D, K Ning, J Li, J Hu, D Han, D Wang, H Wang, X Zeng, X Jing, Q Zhou, X Su, X Chang, A Wang, W Wang, J Jia, L Wei, Y Xin, Y Qiao, R Huang, J Chen, B Han, K Yoon, RT Hill, Y Zohar, F Chen, Q Hu & J Xu. 2014. *Nannochloropsis* genomes reveal evolution of microalgal oleaginous traits. PLOS Genetics 10: 1-13.

Wei L, Y Xin, D Wang, X Jing, Q Zhou, X Su, J Jia, K Ning, F Cheng, Q Hu & J Xu. 2013. *Nannochloropsis* plastid and mitochondrial phylogenomes reveal organelle diversification mechanism and intragenus phylotyping strategy in microalgae. BMC Genomics 14: 534-551.

Zwickl DJ. 2006. Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion. Ph D dissertation, University of Texas, Austin, 115 pp. <http://repositories.lib.utexas.edu/handle/2152/2666>

Received 13 February 2014 and accepted 02 October 2014

Editor: Claudia Bustos D.