Isolation and characterization of a Nannochloropsis salina isolate from Vietnam with potential for biodiesel production

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Introduction

In this study, waste water from shrimp cultivation ponds in the central province of Ninh Thuan, Vietnam was used for the isolation of microalgae. The isolate nl6 was genetically identified as *Nannochloropsis salina* (*N. salina*) using 18S rDNA – ITS sequence. The characterization of isolate *nl6* was performed in term of growth, protein content, fatty acid and pigment profile. The effects of growth phases and salinities on the fatty acid profiles were also studied.

Isolation and identification: A PCR fragment of 2600 bp amplified using the primers NS1 and ITS4 was 100% identical to *Nannochloropsis salina* (D12, accession number JX185299.1) from Shandong in China.

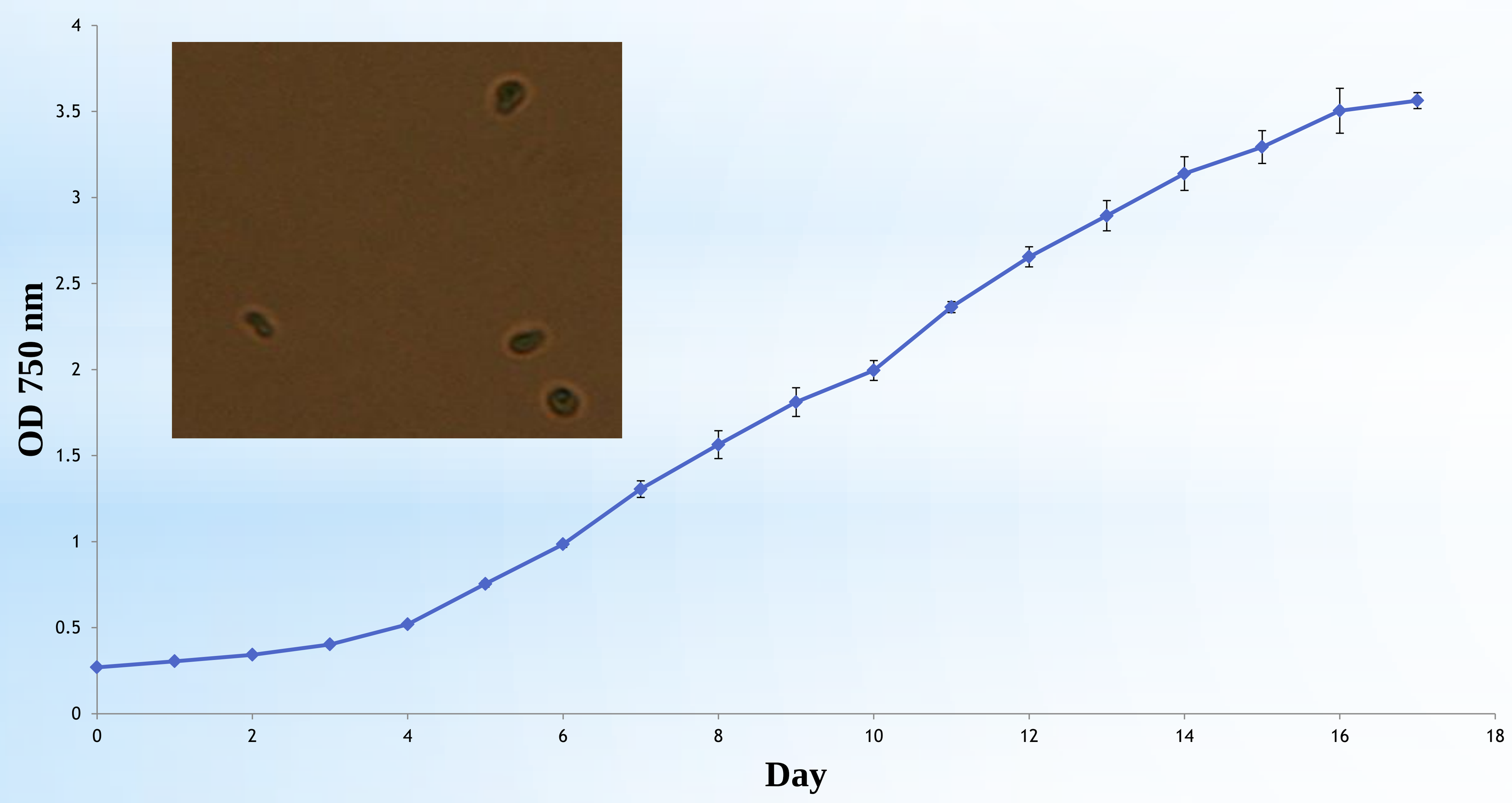
Sequences producing significant alignments:

Select: All None Selected:0

Alignments Download GenBank Graphics Distance tree of results

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Nannochloropsis salina isolate D12 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spac	4802	4802	100%	0.0	100.00%	JX185299.1
Nannochloropsis oceanica strain LAMB2011 chromosome 2	3633	3633	96%	0.0	92.84%	CP038117.1
Nannochloropsis oceanica strain LAMB2011 chromosome 6	3622	3622	96%	0.0	92.76%	CP038132.1
Nannochloropsis oceanica strain LAMB2011 chromosome 14	3616	7233	96%	0.0	92.66%	CP038111.1
Nannochloropsis gaditana strain CCMP526 18S ribosomal RNA gene, complete sequence	3184	3184	66%	0.0	100.00%	KF040086.1

Growth curve of isolate *N. salina nl6* with the highest dried biomass of 0.603 ± 0.07 g L-1 obtained at 17th day (F/2 medium, 24‰ NaCl, 25°C, 50 µmol $m^{-2}s^{-1}$ light intensity)



Effect of growth phases and salinities (10‰, 20‰, 30‰ and 35‰) on fatty acid profile of *N. salina nl6*

Fatty acid profiles and content of *N. salina nl6* in exponential and stationary phases at light intensity of 50 µmol $m^{-2}s^{-1}$

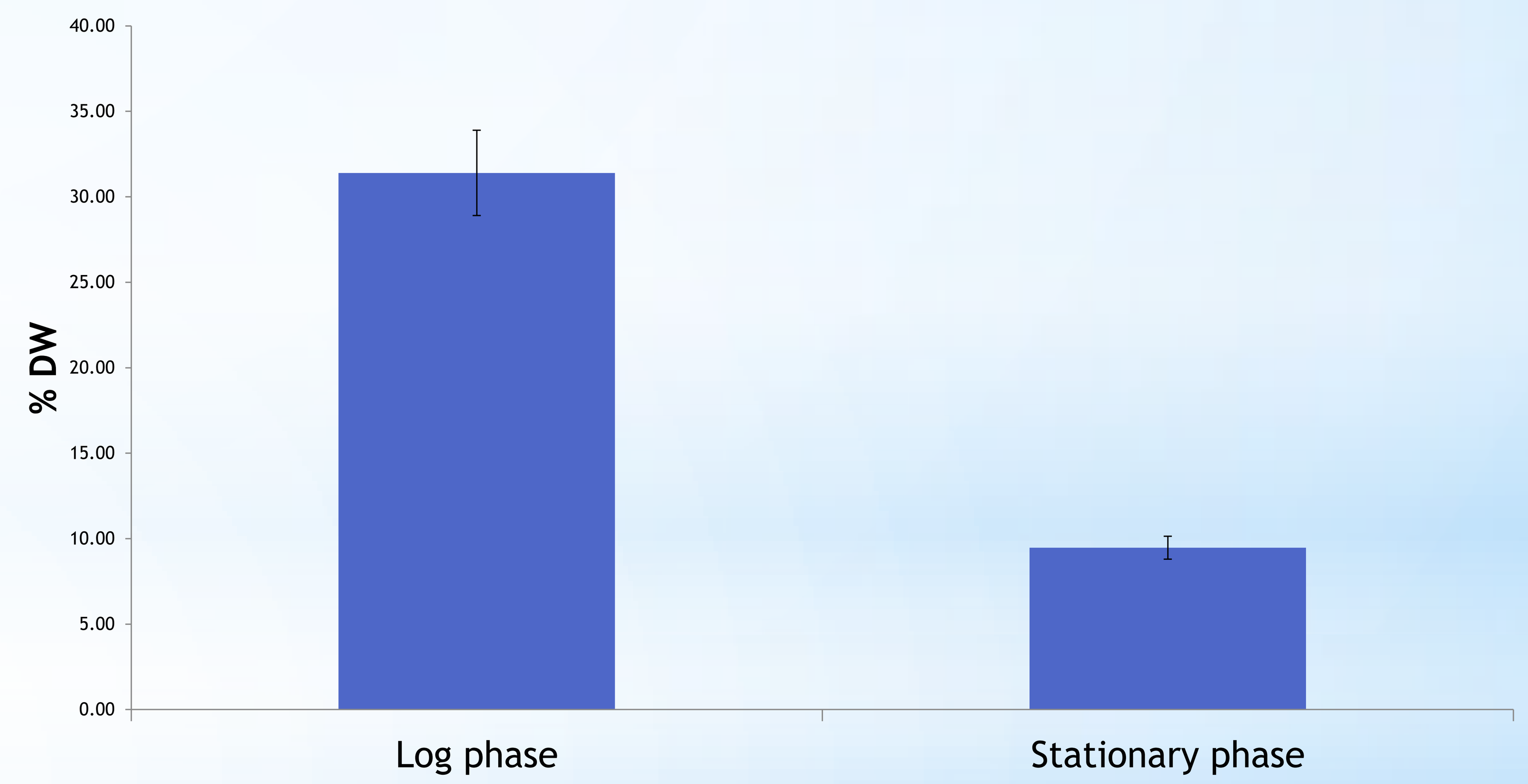
Fatty acid	% total fatty acid	
	Exponential phase	Stationary phase
C_{14}	3.73 ± 0.28	3.15 ± 0.26
C_{16}	33.43 ± 0.28	45.08 ± 2.05
$C_{16:1-cis}$	29.92 ± 1.23	33.13 ± 0.99
C_{18}	5.60 ± 0.12	2.23 ± 0.20
$C_{18:1-cis}$	5.73 ± 1.16	10.13 ± 0.46
$C_{18:2-cis}$	1.16 ± 0.14	0.37 ± 0.17
$C_{20:3}$	1.09 ± 0.29	nd
$C_{20:4}$	6.84 ± 0.69	2.49 ± 0.24
$c_{20:5}$	12.50 ± 1.21	3.43 ± 0.23
Σ SFA	42.76 ± 0.28	50.46 ± 1.85
Σ MUFA	35.65 ± 2.37	43.25 ± 1.45
Σ PUFA	21.59 ± 2.30	6.29 ± 0.45
% Total Fatty acid/ DW	18.65 ± 1.04	18.17 ± 2.67

Fatty acid profiles of *N. salina nl6* in different salinities at light intensity of 216 µM $m^{-2}s^{-1}$

Fatty acid	% TFA			
	10‰	20‰	30‰	35‰
C_{14}	2.81 ± 0.03	2.90 ± 0.01	3.89 ± 0.09	1.94 ± 0.51
C_{16}	48.50 ± 5.25	43.85 ± 2.14	43.86 ± 0.59	44.41 ± 1.35
$C_{16:1-cis}$	40.06 ± 3.99	38.25 ± 1.01	36.37 ± 0.82	37.59 ± 1.58
C_{18}	nd	1.52 ± 0.83	1.24 ± 0.36	0.63 ± 0.16
$C_{18:1-cis}$	8.63 ± 1.28	11.25 ± 0.63	10.88 ± 1.10	10.89 ± 0.29
$C_{18:2-cis}$	nd	0.40 ± 0.04	0.57 ± 0.09	0.18 ± 0.25
$C_{20:3}$	nd	nd	nd	0.01 ± 0.00
$C_{20:4}$	nd	0.97 ± 0.09	1.51 ± 0.38	1.93 ± 0.24
$c_{20:5}$	nd	0.86 ± 0.19	1.68 ± 0.47	2.44 ± 0.31
Σ SFA	51.31 ± 5.28	48.27 ± 1.31	48.99 ± 1.04	46.98 ± 1.00
Σ MUFA	48.69 ± 5.28	49.50 ± 1.65	47.26 ± 0.28	48.47 ± 1.30
Σ PUFA	0.00 ± 0.00	2.23 ± 0.33	3.76 ± 0.76	4.55 ± 0.29

In general, *N. salina nl6* was considered as a potential feedstock for biodiesel production as all the fatty acid profiles were rich in fatty acid with chain lengths in the range of C_{16} to C_{18}, including C16:0, C16:1, C18:0, and C18:1 (Knothe, 2009). SFA and MUFA in all salinities and growth phases were predominant.

Protein content decreased from 31.40 ± 2.50 in exponential phase to 9.47 ± 0.67 % DW in stationary phase.



Pigment profiles in exponential and stationary phase

Pigment	(mg/g DW)	
	Exponential phase	Stationary phase
Lutein	0.48 ± 0.02	0.22 ± 0.03
Neoxanthin	0.13 ± 0.00	0.00 ± 0.00
Violaxanthin	2.51 ± 0.11	0.59 ± 0.10
Anthéraxanthin	0.00 ± 0.00	0.00 ± 0.00
Chl b	0.00 ± 0.00	0.00 ± 0.00
Chl a	12.20 ±0.49	3.07 ± 0.36
B-carotene	0.27 ± 0.00	0.06 ± 0.01
Astaxanthin	0.00 ±0.00	0.36 ± 0.03
Canthaxanthin	0.00 ± 0.00	0.18 ± 0.01

Chlorophyll a, lutein, violaxanthin, neoxanthin and beta carotene contents decreased dramatically in stationary phase while two valuable pigments astaxanthin and canthaxanthin increased in stationary phase. The content of astaxanthin and cantaxanthin were detected at 0.36 ± 0.03 mg/g DW and 0.18 ± 0.01 mg/g DW

Conclusion

A microalgal strain was isolated and genetically identified as *N. salina nl6* based on rDNA – ITS sequence. The strain was proved to be potential for biodiesel production with high biomass content of 0.603 ± 0.07 g L^{-1} and fatty acid content of 18.17 ± 2.67% DW. With the presence of astaxanthin and canthaxanthin in stationary phase, *N. salina nl6* could be a promising candidate for the co-production of biodiesel and valuable pigments.

References

Knothe, G., 2009. Improving biodiesel fuel properties by modifying fatty ester composition. Energy Environ. Sci. 2, 759–766.

Acknowlegements

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