Isolation of new microalgal strains from the Gulf of Patras (Greece) and investigation of their potential biotechnological applications

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Introduction

Microalgae are phytoplankton with worldwide spread and are considered as biological material of high importance, due to their ability to synthesize metabolites of high-added value, such as lipids (often rich in polyunsaturated fatty acids, PUFAs), proteins and carbohydrates in significant quantities [1-3]. The **aim** of this study was the isolation of new microalgal strains from the Gulf of Patras (Greece), their molecular identification and biochemical characterization.

Methods

- **Sample collection:** selected sites along the Gulf of Patras (Fig. 1).
- **Molecular identification:** PCR amplification of the 18S rRNA gene and the ITS (internal transcribed spacer) region.
- **Culture conditions:** batch cultures in modified Artificial Sea Water (ASW) in (a) 0.5 L Erlenmeyer flasks and (b) a 3.7 L Stirred Tank Reactor (STR) (Fig. 2).
- **Cell growth & biomass determination:** cell enumeration and harvesting by centrifugation.
- **Lipids extracted** and converted into their fatty acid methyl-esters for **fatty acid composition analysis** in Gas Chromatography as described elsewhere [4].
- **Determination of cellular proteins & carbohydrates:** biuret method and hydrolysis method with HCl, respectively [4,5].

Results

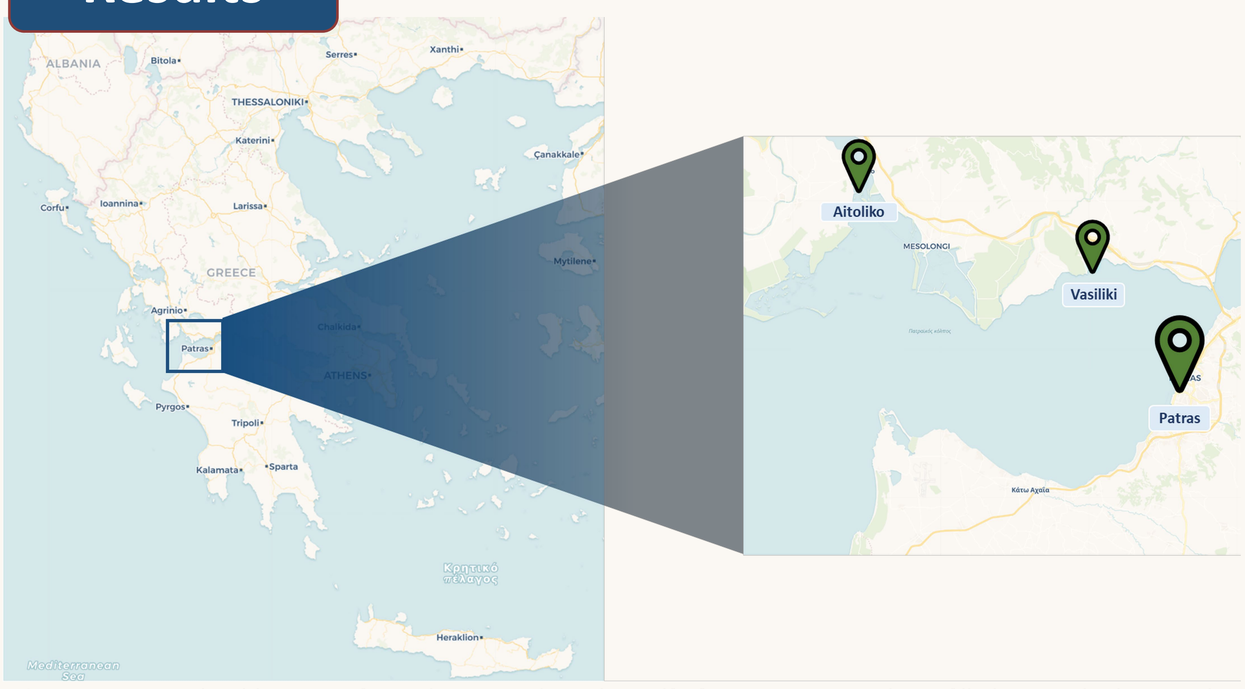

Figure 1. Geographical location of sampling sites along the Gulf of Patras, Greece. The unfilled rectangle denotes the isolation sites which are marked on the right part of the figure. Map data: © OpenStreetMap contributors © CARTO.

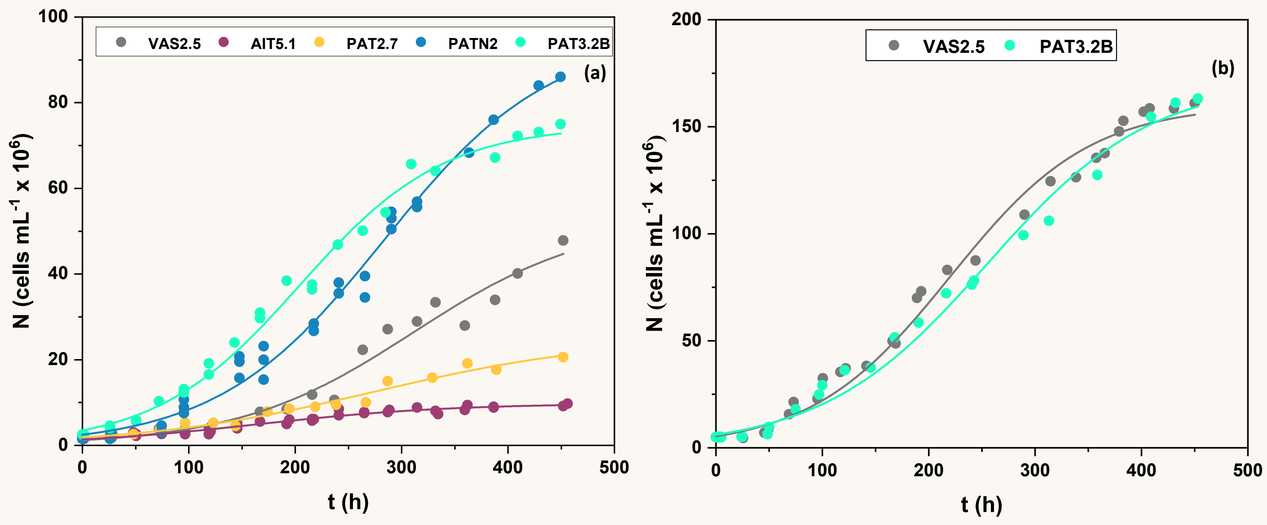

Figure 3. Growth curves of the isolated strains of microalgae cultivated in: (a) Erlenmeyer flasks 0.5-L and (b) Stirred Tank Reactor. **Culture conditions:** ASW; pH = 8.5 ± 0.5; temperature 26.0 ± 1.0 °C; aeriation 0.5 vvm; photoperiod 24:0; illumination (a) 387 μmol m^{-2} s^{-1} and (b) 1071 μmol m^{-2} s^{-1}; agitation rate (a) periodical shaking and (b) 100 rpm.

The six isolated strains belong to the genera *Picochlorum*, *Nannochloropsis* and *Nephroselmis* (Table 1). Most of the strains exhibited adequate growth when cultivated in 0.5-L Erlenmeyer flasks (Fig. 3). Notably, *Nannochloropsis* sp. PATN2 produced >900 mg L^{-1} of dry biomass, while *P. costavermella* VAS2.5 stood out in lipid accumulation (i.e., 19% w/w). The fatty acids of *Picochlorum* and *Nannochloropsis* strains contained significant quantities of PUFAs (mainly a-linolenic or eicosapentaenoic acid), while those of *N. pyriformis* PAT2.7 were suitable for biodiesel manufacture (Table 2). All strains accumulated considerable amounts of proteins and carbohydrates. Additionally, both of the *Picochlorum* strains were cultured in the STR. Biomass production was 2-3 times higher and slight differences were observed regarding storage material accumulation (Table 1).

Table 1. Dry-biomass production (x, mg L^{-1}) and intracellular lipid (L/x%, w/w), carbohydrates (C/x %, w/w) and proteins (P/x%, w/w) accumulation in x. (a) 0.5-L Erlenmeyer flasks, (b) Stirred Tank Reactor 3.7 L.

	Microalgal strain	Origin	x (mg L^{-1})	L/x (%)	C/x (%)	P/x (%)
a	*P. costavermella* VAS2.5	Vasiliki. Aetolia-Acarnania	392.1 ± 2.3	19.1 ± 0.1	5.0 ± 0.2	33.0 ± 5.7
	N. gaditana AIT5.1	Aitoliko. Aetolia-Acarnania	204.6 ± 36.3	12.8 ± 4.4	13.4 ± 1.1	19.2 ± 2.7
	Nannochloropsis sp. PATLG-N1	Patras. Achaia	639.4 ± 22.8	11.1 ± 1.2	10.4 ± 0.0	21.9 ± 0.7
	P. oklahomense PAT3.2B	Patras. Achaia	320.3 ± 55.1	10.3 ± 2.2	7.2 ± 2.1	43.4 ± 1.4
	N. pyriformis PAT2.7	Patras. Achaia	628.5 ± 56.2	4.2 ± 1.2	13.4 ± 1.6	46.8 ± 2.4
	Nannochloropsis sp. PATN2	Patras. Achaia	959.5 ± 11.2	11.1 ± 0.0	9.1 ± 0.1	22.5 ± 5.8
b	*P. costavermella* VAS2.5	Vasiliki. Aetolia-Acarnania	1266.9 ± 84.7	19.1 ± 0.8	13.5 ± 0.8	25.0 ± 0.2
	P. oklahomense PAT3.2B	Patras. Achaia	638.6 ± 53.3	11.5 ± 0.3	11.2 ± 1.2	50.6 ± 3.0

Table 2. Fatty acid composition of total lipid of the isolated microalgae strains at the end of their culture in 0.5-L Erlenmeyer flasks. Experiments were performed in duplicate.

Strain name	Fatty acid composition (w/w%) of total lipid												
	C14:0	$^{\Delta 9}$C14:1	C16:0	$^{\Delta 9}$C16:1	C17:0	C18:0	$^{\Delta 9}$C18:1	$^{\Delta 9,12}$C18:2	$^{\Delta 9,12,15}$C18:3	$^{\Delta 6,9,12,15}$C18:4	$^{\Delta 13}$C20:1	$^{\Delta 5,8,11,14,17}$C20:5	Others
VAS2.5	8.9	6.2	28.5	38.9	0.8	1.0	10.1	2.3	0.2	2.3	ND	ND	0.9
AIT5.1	1.1	5.4	18.7	10.3	ND	1.7	15.1	15.0	15.4	ND	< 0.1	5.1	12.2
PATLG-N1	2.9	1.4	26.1	40.2	ND	0.3	10.5	1.4	ND	ND	3.5	10.7	3.0
PAT3.2B	0.9	5.6	13.1	5.1	4.9	1.4	22.1	16.4	18.2	2.8	ND	< 0.1	9.6
PAT2.7	28.8	4.8	9.3	38.8	0.4	4.5	7.0	0.7	ND	ND	ND	ND	5.8
PATN2	3.6	1.1	26.4	33.2	ND	2.0	13.7	3.0	0.1	ND	3.0	11.8	2.1

Others: C10:0, C12:0, C16:2, $^{\Delta 6,9,12}$C18:3 | Abbreviations: ND, not detected

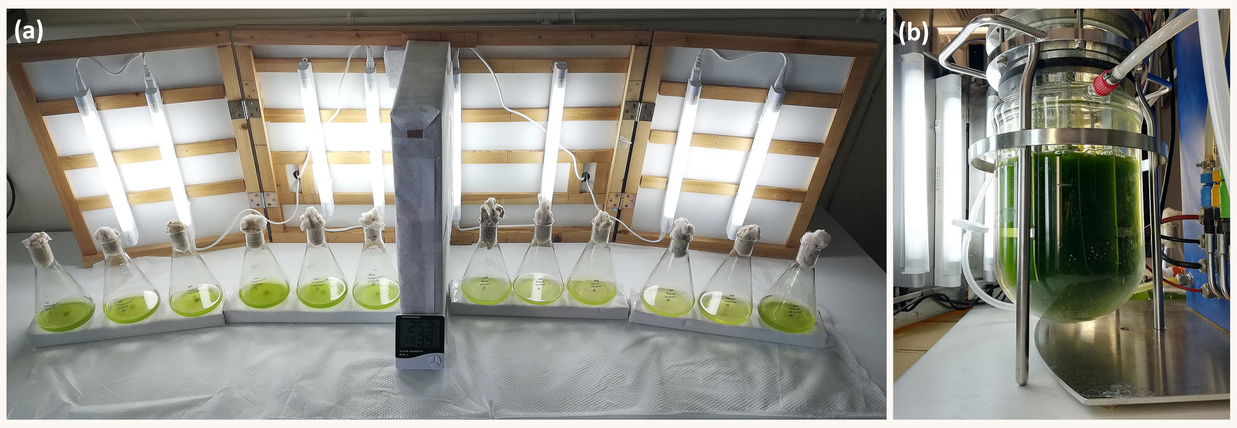

Figure 2. Bioreactors used in this investigation: (a) Erlenmeyer flasks 0.5-L (V_w = 0.1 L) and (b) STR 3.7 L (V_w = 2.0 L). V_w: working volume.

Conclusions

The biochemical profiles of the isolates showcased their potential suitability in various industrial applications, such as in aquaculture, which is a sector of crucial importance for the economy of many countries located in the Mediterranean Sea.

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IonianAlgae

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