

Supplementary Information

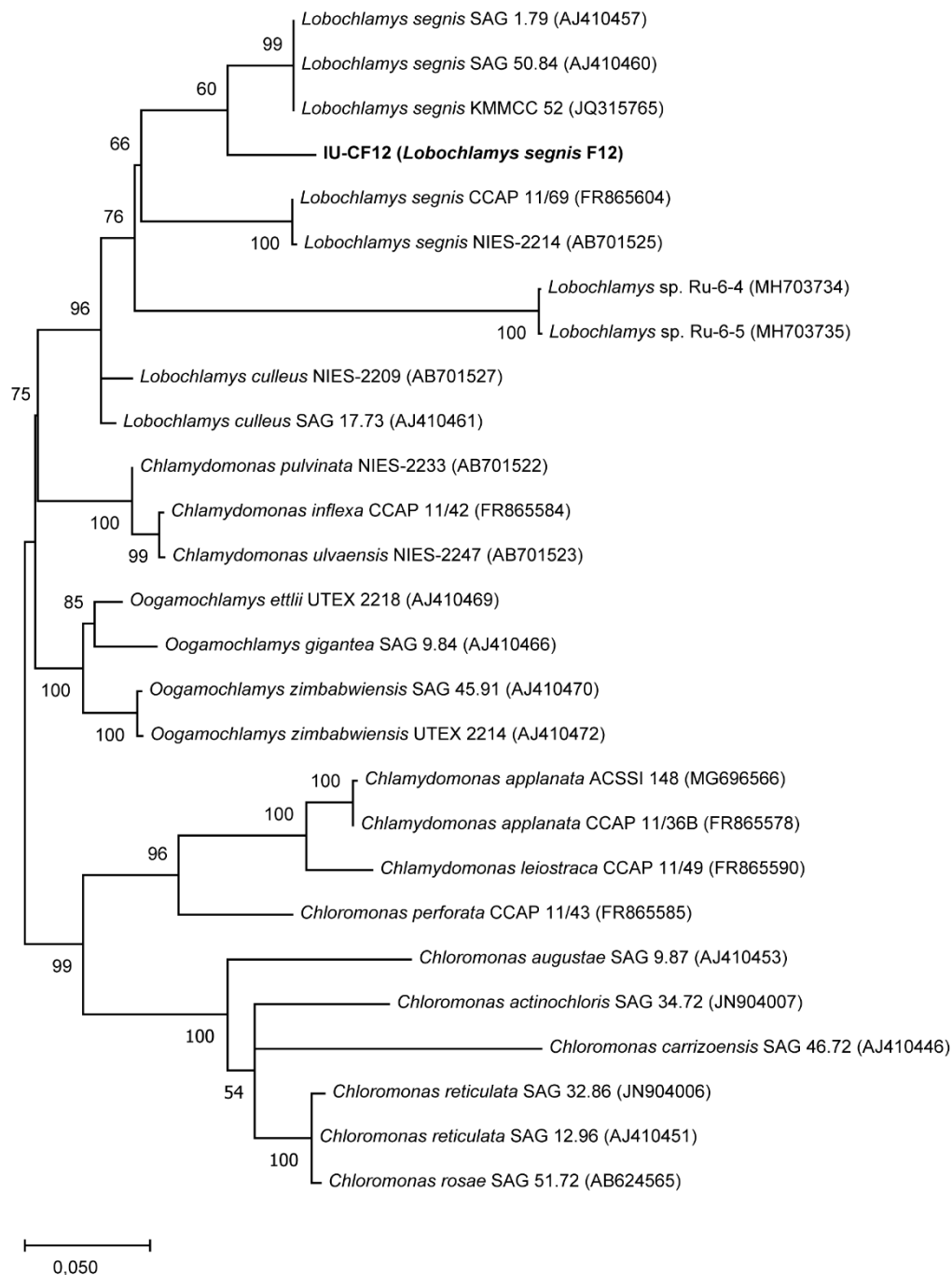
Semi-continuous cultivation for enhanced protein production using indigenous green microalgae and synthetic municipal wastewater

Ikumi Umetani ¹, Michał Sposób ¹ and Olga Tiron ²

¹ Norwegian Institute of Bioeconomy Research, Norway

² National Research and Development Institute for Industrial Ecology, Romania

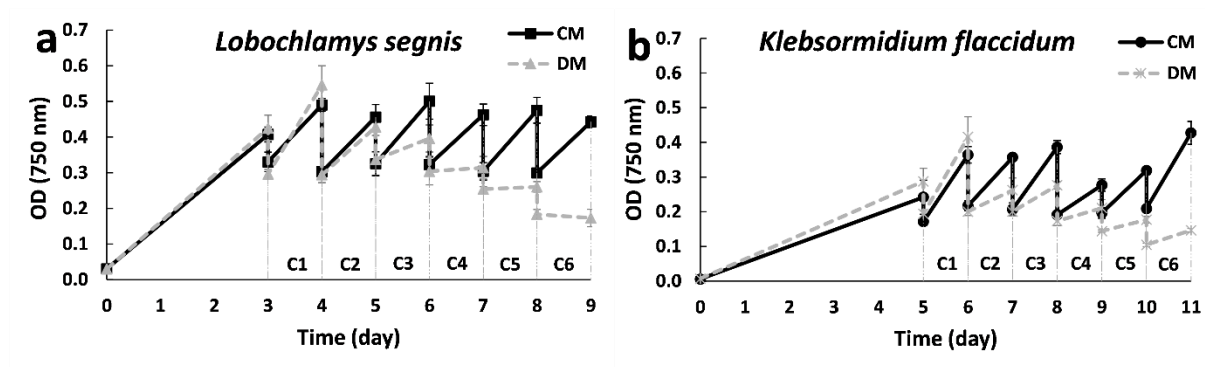
Corresponding author Email: ikumi.umatani@nibio.no



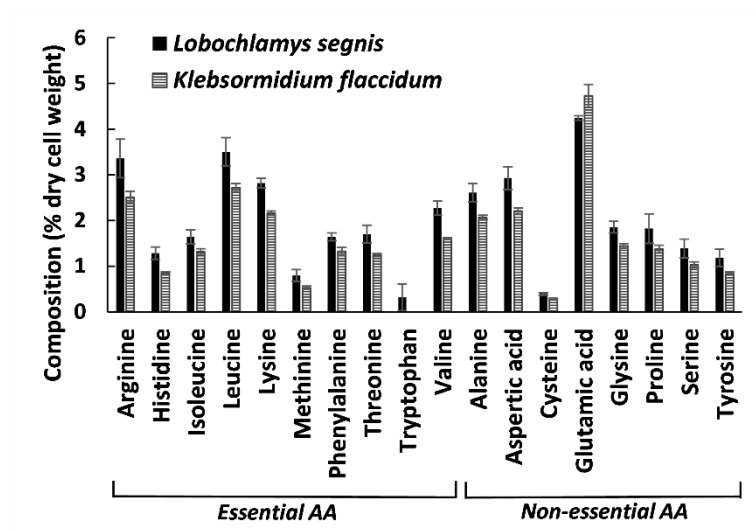
Online Resource Fig. 1 Molecular phylogeny of *Lobocholamys segnis* F12 (shown as IU-CF12) and representative strains of Chlamydomonadales based on 18S rDNA sequences. IU-CF12 was nested in the cluster of strains designated *Lobocholamys segnis*. The phylogenetic analysis was conducted using MEGA X. The maximum likelihood tree was constructed using the Tamura-Nei model with a discrete gamma distribution and proportion of invariable sites (TN+G+I) and 1000 bootstrap replicates. The sequence data for *L. segns* F12 is available in GenBank with the accession number MT973497 (18S rDNA) and MT968751 (ITS1-5.8S rDNA-ITS2)

Online Resource Table 1 Measurement of optical density (OD) and the number of cells ($\times 1000$ cells mL^{-1}) at the start of each cultivation cycle for *Lobochlamys seignis* and *Klebsormidium flaccidum* culture. The OD was measured at 750 nm, and the cell count was conducted using Neubauer-improved hemocytometer (Assistant, Sondeheim, Germany). Mean values ± 1 SD of 3 replicate cultures are shown (3 measurements from each)

Cultivation cycle	<i>Lobochlamys seignis</i>		<i>Klebsormidium flaccidum</i>	
	OD	Cell count ($\times 1000$ cells mL^{-1})	OD	Cell count ($\times 1000$ cells mL^{-1})
1	0.33 ± 0.03	330.2 ± 24.5	0.17 ± 0.01	174.2 ± 49.8
2	0.30 ± 0.01	371.9 ± 88.8	0.22 ± 0.02	210.2 ± 46.5
3	0.33 ± 0.03	371.9 ± 34.1	0.21 ± 0.02	208.8 ± 23.8
4	0.32 ± 0.02	412.4 ± 38.8	0.19 ± 0.01	142.6 ± 64.3
5	0.30 ± 0.02	424.4 ± 1.9	0.20 ± 0.02	204.3 ± 44.4
6	0.30 ± 0.01	410.6 ± 21.7	0.21 ± 0.01	203.2 ± 33.5
Average	0.31	386.9	0.20	190.5
SD	0.01	35.5	0.02	27.0
Relative SD	4.3%	9.2%	8.1%	14.2%



Online Resource Fig. 2 Changes in the Optical density (OD) (750 nm) of (a) *Lobochlamys seignis* and (b) *Klebsormidium flaccidum* culture under the semi-continuous cultivation regime using a complete medium (CM, black full line) and a medium deficient in nitrogen and phosphorus (DM, grey dotted line). The OD was measured both at the start and in the end of each cultivation cycle (C1-C6). The commencement of the semi-continuous cultivation regime for *L. seignis* and *K. flaccidum* was on day 3 and day 5, respectively, when their OD level was reached above the predetermined initial OD for the regime (0.3 and 0.2, respectively). Mean values ± 1 SD (error bars) of 3 replicate cultures are shown (3 measurements from each)



Online Resource Fig. 3 Amino acid profiles of (a) *Lobocholamys segnis* (black bar) and (b) *Klebsormidium flaccidum* (grey striped bar) that grown under the semi-continuous cultivation regime with a complete medium. The analysis was conducted using the samples collected at the end of the 6th cycle. The aspartic acid concentration is sum of aspartic acid and asparagine, and the aspartic acid concentration is the sum of glutamic acid and glutamine. Mean values $\pm$ 1 SD (error bars) of 3 replicate cultures are shown (1 measurement from each)