As explained above, new strains with higher real-world triacylglyceride productivity are required that can operate efficiently under the multiple abiotic stresses experienced during culture.

US2014/0199739 (Calleja et al) relates to strains of microalgae belonging to the *Isochrysis* genus, which are reported to produce polyunsaturated fatty acids, notably eicosapentaenoic acid, in mixotrophic mode. This publication further relates to methods for selecting and culturing such strains, using a discontinuous supply of light in the form of flashes. This is intended to permit possible savings in space and energy related to the supply of a weaker light intensity.

US8067225 (Fernandez Sevilla et al.) relates to a strain of *Scenedesmus* microalga species and to the use thereof for animal and/or human consumption and in the production of carotenoids. The strain was isolated at the Las Palmerillas experimental station, Cajamar.

WO2005003309 relates to the culture of isolated pseudopterosin-producing alga of the clade B genus Symbiodinium. Preferred conditions are a 14-10 hour light-dark cycle, and illumination of about 30 up to 150 µmol photons $m^{-2}s^{-1}$ at a temperature from about 20°C to about 30°C.

US2015/0337255 relates to a culture method for microalgae that cultures unicellular green microalgae belonging to the genus *Coccomyxa* and groups of organisms closely related thereto, or the Watanabea clade, in an open outdoor culture system using broth having a pH of 4 or lower. It further relates to a culture method for microalgae of the genus *Pseudococcomyxa*.

It can be seen that the provision of novel microalga species, particularly those with novel adaptations to the environment, would provide a contribution to the art.

Summary

We carried out a bioprospecting survey of the Malacca Straits and South China Sea. Both are distinguished by very high rates of solar radiation, year-round high temperatures with variable/high salinity seawaters (solar radiation 2100 µM photons.s^{-1}.m^{-2}; year-round high temperatures 28°C-30°C; variable high salinity waters 32 -34 PSU). These conditions can provide the optimal marine environment for the evolution of high-light, high-temperature, high-salinity adapted microalgae.

We have developed an efficient and scalable fourth generation marine microalgal biofuel production technology consisting of a suite of multiple isolates of a completely new genus of extremophile marine microalgae we have named *Sphaerica*. These isolates have been engineered using directed-evolution approaches to create highly efficient carbon capture and conversion production strains which can be cultured in large format photobioreactors using atmospheric, compressed, recovered CO_2 or industrial flue gas and combined with appropriate biomass/lipid downstream processing steps to produce useful product classes including carbon-neutral biofuels based on or derived from *Sphaerica* triacylglycerides (“bio-

oil"), polyunsaturated fatty acids (PUFA) and high protein, PUFA enriched, fish and animal feed. In addition, *Sphaerica* can act as effective, sustainable and economic carbon capture decarbonisation agent for industrial flue gases.

In particular, examples are provided of directed evolution engineered *Sphaerica* strains which can utilise high levels of sunlight, temperature and high variable salinity. Therefore, they are capable of highly efficient generation of biomass, bio-oil, PUFA and other biomass-derived products in Tropical and Sub-tropical regions where its growth requirements (light, temperature and salinity) are met. In addition, *Sphaerica* can also be cultured in artificial systems such as using man-made light and heat sources in locations outwith the sub-Tropical/Tropical zone.

Three *Sphaerica* isolates HB001, HB002 and HB003 have been deposited with the Culture Collection of Algae and Protozoa. In addition, 19 other *Sphaerica* spp. have been isolated and characterised. Collectively these represent part of a distinct new genus of unicellular marine microalgal defined by phenotypic and genotypic traits described herein.

In one aspect, the disclosure provides a marine microalgal strain of the Ulvales order of the Ulvophyceae class characterised by at least 5, 6, 7, or 8, or having all, of the following properties: (i) being unicellular and uni-nucleate; (ii) non motile, without cilia nor flagellae; (iii) having generally spherical cells; (iv) having a diameter of about 1.00 to 11.0 µm, more preferably 1.5 µm to 10.3 µM; (v) having an extracellular polysaccharide capsule 0.215 µm to 0.344 µm thick; (vi) having chloroplasts that are parietal and a single pyrenoid; (vii) includes lipid bodies. (viii) capable of asexual reproduction within maternal capsule through two sequential divisions at approximately 90° to each other into 4 nonmotile daughter cells enclosed within their own capsule, daughter cells released by rupture of maternal cell envelope, said strain also: (ix) having from 120 – 300 thylakoid membranes per chloroplast (x) having an 18S rRNA which exhibits 97% or higher base sequence homology with the 18S rRNA sequence shown in Figure 5.

In one aspect, the disclosure provides a microalgal strain characterized in that it consists of an isolated strain deposited at the CCAP under accession number CCAP 2271/1, CCAP 2271/2 or CCAP 2271/3.

In one aspect, the disclosure provides a microalgal strain which strain is a derivative of any of a marine microalgal strain defined in the disclosure having the properties of the microalgae defined in the disclosure, which strain is optionally a directed evolution engineered derivative strain which is capable of more efficient growth in vitamin-free media having a PSU of greater than or equal to 30, 35, 40, or 45 PSU, as compared to the growth rate in otherwise identical low PSU media.

In one aspect, the disclosure provides a cell extract; a cell suspension; a cell homogenate; a cell lysate; a cell pellet, a culture broth, or a cell free or substantially cell free culture broth, in each case of a strain as claimed in the disclosure.

In one aspect, the disclosure provides a process for making a derived strain of the disclosure from the deposited strain of the disclosure or from the screened strain of the disclosure,

which process comprises directed evolution, wherein the directed evolution optionally comprises one or more of the following steps: (i) culturing the deposited parent strain under an environmental stress selected from: high salinity and/or high insolation, wherein the intensity of said stress is increased step-wise; (ii) maintaining said culture over several generations; (iii) isolating the strain(s) for which the number of cells has most increased during said generations.

In one aspect, the disclosure provides a process for making a derived strain of the disclosure from the deposited strain of the disclosure or from the screened strain of the disclosure, which process comprises mutagenesis and selection, wherein the mutagenesis and selection optionally comprises one or more of the following steps: (i) culturing the deposited parent strain under conditions promoting mutagenesis; (ii) maintaining said culture over several generations; (iii) isolating the strain(s) for which the number of cells has most increased during said generations.

In one aspect, the disclosure provides a process for making a derived strain of the disclosure from the deposited strain of the disclosure or from the screened strain of the disclosure by recombinant molecular biology, comprising one or more of gene insertion, deletion, or modification.

In one aspect, the disclosure provides a method of producing microalgal biomass or producing a desired product or compound produced by microalgal culture, which method comprises culturing a population of marine microalgae as claimed in the disclosure .

In one aspect, the disclosure provides the use of a microalgal strain as claimed in the disclosure, or a method of the disclosure, for carbon capture from a CO2 containing gas, which is optionally an industrial flue gas.

Thus in one example the disclosure provides novel marine microalgal strain of the Ulvales order of the Ulvophyceae class characterised by a novel combination of phenotypic and genotypic properties.

Such algal strains can be provided, in the light of the present disclosure, by screening of microalgae or microalgal biofilms from marine environments, for example by using media described herein comprising filtered seawater supplemented with vitamin-free nitrogen sources, phosphorous and trace elements.

In another example there is provided a process for making derived (mutant) strains, for example from parent deposited strains or screened strains.

The disclosure further provides, in relation to marine microalga of the disclosure, methods of culturing, storing, or using said strains e.g. to produce biomass, or produce products.

In another example there is provided a method of producing microalgal biomass or producing a desired product produced by microalgal culture, which method comprises culturing a population of marine microalgae of the disclosure.

Populations of microalgae will collectively have the characteristics described above e.g. an average EPS thickness of around 280 nm etc.

Examples of products include biofuel or biofuel additives comprising microalgal triacylglycerides, polyunsaturated fatty acids (PUFA) and high protein foods or feeds. Additionally the microalgal capsules which are sloughed off after division can be a rich source of sucrose which provides a source of low carbon sugar for fermentations.

The disclosure further provides these products obtained or obtainable from the strain e.g. in isolated or enriched form, or downstream products utilising these microalgal products.

In another example there is provided use of as a microalgal strain or related method of the disclosure for carbon capture from a CO_2 containing gas, which is optionally an industrial flue gas.

Some of these examples and examples will now be described in more detail.

Detailed description of the invention

The present invention relates to novel marine microalgal strains of the Ulvales order of the Ulvophyceae class. This new Chlorophyte genus is termed *Sphaerica* herein.

In support of this invention, three *Sphaerica* isolates HB001, HB002 and HB003 have been deposited May 12 2022 with Culture Collection of Algae and Protozoa as patent strains with the following codes:

CCAP 2271/1 *Sphaerica* sp. HB001
CCAP 2271/2 *Sphaerica* sp. HB002
CCAP 2271/3 *Sphaerica* sp. HB003

Culture Collection of Algae and Protozoa, Scottish Association for Marine Science (SAMS) Ltd. Oban, Argyll PA37 1QA, UK

These deposits are incorporated herein by reference. The isolated strains of the present invention have not previously been made available to the public.

As used herein, HB002 may also be referred to as “HBMSM0012”. HB003 may be referred to as “HBMSM0031”. HB001 may be referred to as “HBMSM0061”.

In addition to HB001, HB002, and HB003,nineteen other *Sphaerica* spp. have been isolated and characterised by the present inventors: HBMSM0011; HBMSM0013; HBMSM0014; HBMSM0015; HBMSM0021; HBMSM0022; HBMSM0023; HBMSM0032; HBMSM0033; HBMSM0034; HBMSM0051; HBMSM0053; HBMSM0054; HBMSM0062; HBMSM0063; HBMSM0064; HBMSM0065; HBMSM0081 and HBMSM0082.

Collectively these deposited strain represent part of a distinct new genus of unicellular marine microalgal defined by the advantageous multigenic traits described herein. Small subunit 18S rRNA sequence data prove the strains are of independent and genetically heterogenous origin (Figure 6). More specifically, the full length HB001, HB002 and HB003 18S rRNA sequences were used as initial inputs for phylogenetic the analysis that generated Figure 6. The *Sphaerica* HB001, HB002 and HB003 18S rRNA sequences are 1797 bp long and were derived from functional annotation of the *Sphaerica* genome sequences of each HB species, in particular by sequence conservation across closely related genera (Chlorophyta defined by Lin, G. M., Lai, Y. H., Audira, G., &; Hsiao, C. der. (2017). A Simple Method to Decode the Complete 18-5.8-28S rRNA Repeated Units of Green Algae by Genome Skimming. International Journal of Molecular Sciences, 18(11). https://doi.org/10.3390/IJMS18112341.

Claims

1 A marine microalgal strain of the Ulvales order of the Ulvophyceae class characterised by at least 5, 6, 7, or 8, or having all, of the following properties:
(i) being unicellular and uni-nucleate;
(ii) non motile, without cilia nor flagellae;
(iii) having generally spherical cells;
(iv) having a diameter of about 1.00 to 11.0 µm, more preferably 1.5 µm to 10.3 µM;
(v) having an extracellular polysaccharide capsule 0.215 µm to 0.344 µm thick;
(vi) having chloroplasts that are parietal and a single pyrenoid;
(vii) includes lipid bodies.
(viii) capable of asexual reproduction within maternal capsule through two sequential divisions at approximately 90° to each other into 4 nonmotile daughter cells enclosed within their own capsule, daughter cells released by rupture of maternal cell envelope,
said strain also:
(ix) having from 120 – 300 thylakoid membranes per chloroplast
(x) having an 18S rRNA which exhibits 97% or higher base sequence homology with the 18S rRNA sequence shown in Figure 5

2 A microalgal strain of claim 1 wherein:
(ai) the chloroplasts exhibit penta-radial symmetry occupying upper cell hemisphere with lobes extending into the remaining hemisphere which contains the lipid bodies; and/or
(aii) the thylakoids enclose an approximately 17 nm wide lumen and form a continuous structure throughout the chloroplast with thylakoids running parallel to the cell membrane; and/or
(aiii) the neutral lipid content is from 5% to 70%, optionally 5% to 65%, of cell dry mass, and/or the microalgal strain
(bi) has higher growth rates when cultured externally on a 12:12 day/night cycle having a maximum daytime insolation of greater than or equal to 1600 µM photons $s^{-1}.m^{-2}$ as compared to a maximum of 150, 200, 250, or 300 µM photons $s^{-1}.m^{-2}$; and/or
(bii) remains viable after 72-hours culture at 45ºC;
and/or said strain
(c) is capable of producing the compounds shown in Table 2 and optionally Table 3..

3 A microalgal strain characterized in that it consists of an isolated strain deposited at the CCAP under accession number CCAP 2271/1, CCAP 2271/2 or CCAP 2271/3.

4 A microalgal strain as claimed in any one of claims 1 to 2 which strain is obtained or obtainable by screening from a marine environment using filtered seawater supplemented with vitamin-free nitrogen sources, phosphorous and trace elements,which strain is optionally present in the same phylogenetic clade within the Ulvophyceae as a strain deposited under accession number CCAP 2271/1, CCAP 2271/2 or CCAP 2271/3.

5 A microalgal strain which strain is a derivative of any of a marine microalgal strain defined in any one of claims 3 to 4 having the properties of the microalgae defined in any one of claims 1 to 2, which strain is optionally a directed evolution engineered derivative strain which is capable of more efficient growth in vitamin-free media having a PSU of

greater than or equal to 30, 35, 40, or 45 PSU, as compared to the growth rate in otherwise identical low PSU media.

6 A strain as claimed in any one of claims 1 to 5 which is in the form of
(i) a biologically pure culture of said strain, which may be a slope culture or liquid medium broth, or
(ii) a freeze dried sample, a liquid nitrogen frozen sample, or a frozen preparation in glycerol of said strain.

7 A cell extract; a cell suspension; a cell homogenate; a cell lysate; a cell pellet, a culture broth, or a cell free or substantially cell free culture broth, in each case of a strain as claimed in any one of claims 1 to 6.

8 A process for making a derived strain of claim 5 from the deposited strain of claim 3 or from the screened strain of claim 4, which process comprises directed evolution, wherein the directed evolution optionally comprises one or more of the following steps:
(i) culturing the deposited parent strain under an environmental stress selected from: high salinity and/or high insolation, wherein the intensity of said stress is increased step-wise;
(ii) maintaining said culture over several generations;
(iii) isolating the strain(s) for which the number of cells has most increased during said generations.

9 A process for making a derived strain of claim 5 from the deposited strain of claim 3 or from the screened strain of claim 4, which process comprises mutagenesis and selection, wherein the mutagenesis and selection optionally comprises one or more of the following steps:
(i) culturing the deposited parent strain under conditions promoting mutagenesis;
(ii) maintaining said culture over several generations;
(iii) isolating the strain(s) for which the number of cells has most increased during said generations.

10 A process for making a derived strain of claim 5 from the deposited strain of claim 3 or from the screened strain of claim 4 by recombinant molecular biology, comprising one or more of gene insertion, deletion, or modification.

11 A method of producing microalgal biomass or producing a desired product or compound produced by microalgal culture, which method comprises culturing a population of marine microalgae as claimed in any one of claims 1 to 6.

12 A method as claimed in claim 11 wherein said marine microalgae are cultured:
(ai) at a temperature of between about 20°C to about 50°C, preferably 28°C – 35°C; and/or
(aii) at a pH of between 6 and 11, preferably about pH 8; and/or
(aiii) a salinity of between 15 to 63 PSU, preferably 30 to 40 PSU; and/or
(aiv) a photonic intensity of up to 2100 µmol photons $m^{-2}.s^{-1}$ Photosynthetically Available Radiation, and/or wherein the marine microalgal cells
(b) are mixed during culture via air bubbling into the culture media and\or are supplemented with CO_2, which is optionally industrial flue gas,
and/or the marine microalgae are cultured

(c) externally under natural day/night cycle, and wherein:
(ci) the maximum light intensity in the peak day part of the cycle is at least or about 100 to 2100 µmol photons $m^{-2}.s^{-1}$ at peak daytime exposure; and\or
(cii) the maximum temperature at the peak day part of the cycle is between 30°C to about 50 °C, optionally at least 35°C, 38 °C, 48°C; and\or
(ciii) the maximum salinity during culture is at least or about 30, 35, 40, 45, 50, 55, 60 or 63PSU, and/or
(d) the culture is photoautotrophic.

13 A method as claimed in any one of claims 11 to 12 wherein the culture is carried out in a culture medium which is a synthetic medium prepared from:
(i) either (ia) a natural source of salt-water, which optionally sea water, brackish water, or a salt-lake water, or (ib) an industrial brine product;
(ii) an additional source or sources of a nitrogen containing compound, which is optionally nitrate or urea, and phosphate;
(iii) optionally an additional source of trace elements is provided which optionally comprises one or more of: $FeCl_3$, $CuSO_4$, $ZnSO_4$, $CoCl_2$, $MnCl_2$, or Na_2MoO_4; and
(iv) preferably no added vitamins nor added antibiotic nor antifungal supplements,
optionally wherein the media comprises filtered sea water supplemented with $NaNO_3$ about 7mM; $NaH_2PO_4.2H_2O$ about 0.3 mM; $C_{10}H_{14}N_2Na_2O_8 \cdot 2H_2O$ about 20 µM; $FeCl_3 \cdot 6H_2O$ about 25 µM; $CuSO_4.5H_2O$ about 29 nM; $ZnSO_4.7H_2O$ about 150 nM; $CoCl_2.6H_2O$ about 150 nM; $MnCl_2.4H_2O$ about 2µM; $Na_2MoO_4.2H_2O$ about 50 nM.

14 A method as claimed in any one of claims 11 to 13 wherein the method is performed:
(ai) as batch culture; or
(aii) continuous culture,
wherein optionally the method is performed:
(bi) in an open pond, or
(bii) in a culture reactor, which is optionally a vertical culture reactor or a photobioreactor, which is optionally an air lift column or flat panel photobioreactor.

15 A method as claimed in any one of claims 11 to 14 wherein the method is for producing a desired compound selected from:
(i) a biofuel comprising microalgal triacylglycerides; and/or
(ii) polyunsaturated fatty acids (PUFA) and; and/or
(iii) high protein, PUFA rich food or fish- or animal- feed.

16 A method as claimed in any one of claims 11 to 15 which further comprises the step of recovering, isolating, purifying, or enriching the desired product from the culture, wherein the desired product is optionally derived from the supernatant of the culture, optionally by one or more of centrifugation, filtration or decanting, followed optionally by precipitation, optionally wherein the filtration is r tangential flow filtration.

17 A method as claimed in claim 16 for producing a triacylglyceride composition from the culture, which method comprises solvent extraction of said triacylglycerides optionally comprising the steps of:
(a) filtering the cell mass from the culture;
(b) lysing the algal cells in the cell mass in the presence of a polar-solvent, which is optionally methanol;

(c) mixing the polar-solvent and lysed cellular material with a non-polar solvent which is optionally hexane;
(d) recovering the triacylglyceride-containing non-polar solvent layer;
(e) recovered the triacylglyceride from the non-polar solvent layer.

18 Use of a microalgal strain as claimed in any one of claims 1 to 5, or a method of any one of claims 11 to 14, for carbon capture from a CO2 containing gas, which is optionally an industrial flue gas.