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# Isolation, characterization, and maintenance of native Swiss microalgae for biotechnological prospection

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**Microalgae culture collections may contain unexplored strains with great biotechnological potential. Through sampling, identification, characterization, and maintenance of local strains, part of the work described here led to the establishment of the first public Swiss microalgae culture collection, AlgoScope. The potential biotechnological applications of 7 strains from among over 120 native strains were suggested based on growth parameters and biochemical composition. Under standardized growth conditions, *Tetradesmus obliquus* FAM 27852 and FAM 27855, *Chloroidium saccharophilum* FAM 27962, *Chlorella vulgaris* FAM 27965, *Stichococcus* sp. FAM 27986, *Desmodesmus* sp. FAM 28090, and *Tetranephris brasiliensis* FAM 28097 had growth rates of 0.24 $d^{-1}$–0.80 $d^{-1}$ and biomass productivities of 0.24 g $L^{-1}$ $d^{-1}$–0.73 g $L^{-1}$ $d^{-1}$. Proteins, lipids, carbohydrates, and ashes ranged from 32.88 to 53.54%, 9.69–18.08%, 9.32–23.94%, and 3.17–5.51%, respectively. All strains had a similar amino acid composition, containing all essential amino acids. In contrast, the fatty acid composition varied among strains, but, in general, the fatty acids were rich in PUFAs (23.83–53.49% of total fatty acids). Overall, *C. saccharophilum* FAM 27962 and *T. brasiliensis* FAM 28097 showed great potential for use in the animal feed sector.**

**Keywords** Switzerland, Culture collection, Microalgae biotechnology, Biochemical composition

Microalgal culture collections are a valuable source of genetic and biological material for research and biotechnology purposes. Local collections often contain microorganisms from unique geographic regions and inhospitable environments that require extensive sampling[1]. Moreover, local strains are more suitable for biotechnological use because they are well adapted to local climatic conditions and do not pose an ecological risk as invasive species[2]. In Europe, the interest and investment in microalgae biotechnology has grown rapidly in recent years. Although the geoclimatic conditions are not optimal for outdoor algal growth in a major portion of the European territory, approximately 450 facilities spread over 23 countries produce more than 300 tons of microalgae per year, including cyanobacteria[3,4]. Surprisingly, only a few dozen strains are in commercial use, which is a waste of the great diversity of microalgae[5]. Most of the strains produced typically come from commercial collections, often originating in tropical and subtropical regions, thus making them better adapted to such climates. Therefore, the physiological diversity and metabolic plasticity of microalgae are underestimated in their current biotechnological exploitation; culture collections may hold the solution for this problem.

Microalgae stand out as promising bioresources for a variety of applications, such as food, feed, energy, cosmetics, pharmaceuticals, and natural pigments[6–8]. Due to their morphological, physiological, and metabolic diversity, different strains of microalgae can be found in virtually every type of environment[9]. Ultimately, the potential applications of a given microalgal strain can be inferred from its growth and biochemical characteristics[10]. For example, microalgae are known to contain significant amounts of high-quality proteins (30–70% of the dry biomass) and are considered excellent alternative protein sources; hence, they are interesting for the food and feed sectors[11]. Similarly, microalgal lipids have been extensively studied for food and feed applications or biodiesel production, depending on their fatty acid profile[12]. Pigments are also used in the food sector as colorants and in the pharmaceutical sector mainly as antioxidants. In particular, microalgae have unique pigments compared to plants, such as the carotenoids loroxanthin, astaxanthin, and canthaxanthin[13]. In 2020,

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the algae sector was worth approximately USD 2.5 billion and supported more than 25,000 jobs, consolidating itself as a crucial part of the future blue bioeconomy[14].

Switzerland is a wealthy and industrially mature European country, particularly known for its dairy, pharmaceutical, and animal husbandry sectors. In line with the United Nations Sustainable Development Goals (UN SDGs), the Swiss industry is committed to introducing novel and more sustainable processes into its value chain, such as microalgal biotechnology. For these and other reasons mentioned above, this study aimed to sample, identify, characterize, and maintain native Swiss strains for biotechnological prospection. In particular, one of the results of this study is the development of the first public Swiss microalgae culture collection, which is called AlgoScope. To the best of the authors' knowledge, this is the first initiative of its kind in Switzerland. In the present study, we report the growth parameters and biochemical composition (i.e., proteins, lipids, carbohydrates, ashes, pigments, and amino acids and fatty acids profiles) of 7 out of more than 120 strains isolated in Swiss territory. These strains were selected for the following reasons: (i) they collectively form a representative sample of species commonly found in natural Swiss environments; (ii) they demonstrate the physiological and biochemical diversity among species and strains of the Swiss photosynthetic microbiota; and (iii) most of the species are well-described in the literature. Based on their growth and composition, biotechnological applications were suggested. This work represents a preliminary screening, which is fundamental and needs to be supported by scale-up and implementation feasibility tests.

## Results

### Cell growth and biomass production

To date, over 120 native Swiss microalgae strains were isolated from widely diverse conditions and environments and added to AlgoScope. Seven fully characterized strains of green microalgae (Chlorophyta) that demonstrated the intra- and inter-specific variability of samples were selected and described in this study: *Tetradesmus obliquus* (FAM 27852 and FAM 27855), *Chloroidium saccharophilum* (FAM 27962), *Chlorella vulgaris* (FAM 27965), *Stichococcus* sp. (FAM 27986), *Desmodesmus* sp. (FAM 28090), and *Tetranephris brasiliensis* (FAM 28097) (Fig. 1).

In batch growth under standardized conditions, most strains showed an adaptation (lag) phase that lasted 1–4 days before growing exponentially, being more pronounced in *C. saccharophilum* FAM 27962 and *T. brasiliensis* FAM 28097. Then, after 3–5 days of exponential growth, the cell division rate dramatically reduced as cells reached a stationary phase after 9–10 days (Fig. 2). The specific growth rate varied from 0.80 $d^{-1}$ to 0.41 $d^{-1}$, with the highest growth rate found in *Desmodesmus* sp. FAM 28090 (not significantly different from *T. brasiliensis* FAM 28097; $p < 0.05$) and the lowest in *C. vulgaris* FAM 27965 cultures (not significantly different from *C. saccharophilum* FAM 27962; $p < 0.05$). The highest biomass concentration and productivity (6.27 g $L^{-1}$ and 0.73 g $L^{-1}$ $d^{-1}$, respectively) were observed in *T. brasiliensis* FAM 28097 cultures and were not significantly different from *T. obliquus* FAM 27955, *Desmodesmus* sp. FAM 28090, and *C. saccharophilum* FAM 27962 ($p < 0.05$). The lowest biomass concentration and productivity were found in *C. vulgaris* FAM 27965 cultures (2.42 g $L^{-1}$ and 0.24 g $L^{-1}$ $d^{-1}$, respectively; Table 1).

### Biochemical composition

Overall, the macromolecular composition of dried microalgal biomasses had 32.88–53.54% proteins, 9.69–18.08% lipids, 9.32–23.94% carbohydrates, and 3.17–8.19% ashes. Chlorophylls and carotenoids ranged from 10.21 mg $g^{-1}$–32.08 mg $g^{-1}$, and 1.65 mg $g^{-1}$–6.03 mg $g^{-1}$, respectively (Table 2). The highest contents of proteins, lipids, and ashes in the dry biomass were found in *T. brasiliensis* FAM 28097, whereas the highest content of carbohydrates was observed in *Stichococcus* sp. FAM 27986. The lowest contents of proteins, lipids, ashes, and carbohydrates in the dry biomass were found in *Stichococcus* sp. FAM 27986, *Desmodesmus* sp. FAM 28090, *Stichococcus* sp. FAM 27986, and *T. brasiliensis* FAM 28097, respectively. The latter had also the lowest pigment content in its biomass, whereas *C. saccharophilum* FAM 27962 had the highest, showing similar values as those for *Stichococcus* sp. FAM 27986 ($p < 0.05$; Table 2).

Further, the amino acid composition of the investigated microalgae revealed that the abundance of a given amino acid compared to the others and their absolute values were rather similar between strains (Table 3). All essential and non-essential amino acids were detected in all strains, and their total concentrations were, on average, 40.9% and 50.1% of total protein content, respectively. Among the essential amino acids, leucine and histidine were the most and least abundant, respectively, with leucine having an average concentration of 8.92% and histidine of 1.92% of total proteins. In addition, lysine, phenylalanine, threonine, and valine presented intermediate concentrations of approximately 5–6% of total protein content. Glutamine was the most abundant non-essential amino acid in all strains and, unusually, showed a higher value in *C. saccharophilum* FAM 27962 (18.16%) when compared to that in the other strains (11.42% on average). Similarly, the concentration of the non-essential amino acid arginine was higher in *T. brasiliensis* FAM 28097 (11.12%) than that observed in other strains (6.68% on average). The least abundant non-essential amino acid was cysteine, with an average of 1.62% of total protein content in the microalgae biomass (Table 3).

In contrast with amino acids, the composition of fatty acids varied substantially between strains (Table 4). Fatty acids accounted for 11.01% of the biomass dry weight on average, ranging from 8.68% in *T. obliquus* FAM 27852 to 15.93% in *T. brasiliensis* FAM 28097. *Stichococcus* sp. FAM 27986 had the highest concentration of saturated fatty acids (SFAs) and monounsaturated fatty acids (MUFAs) in the total fatty acid content (19.84% and 35.57%, respectively). In contrast, the lowest concentrations of SFAs and MUFAs were observed in *T.brasiliensis* FAM 28097 (10.39%) and *C. saccharophilum* FAM 27962 (7.00%), respectively. Additionally, polyunsaturated fatty acids (PUFAs) were more abundant in *C. saccharophilum* FAM 27962 (53.49%), with a remarkable contribution of linoleic acid (C18:2*n*−9; 43.74% of total fatty acids). Even though linoleic acid was the most abundant PUFA in all strains, all values were considerably lower than that (Table 4). Palmitic acid was the most abundant SFA

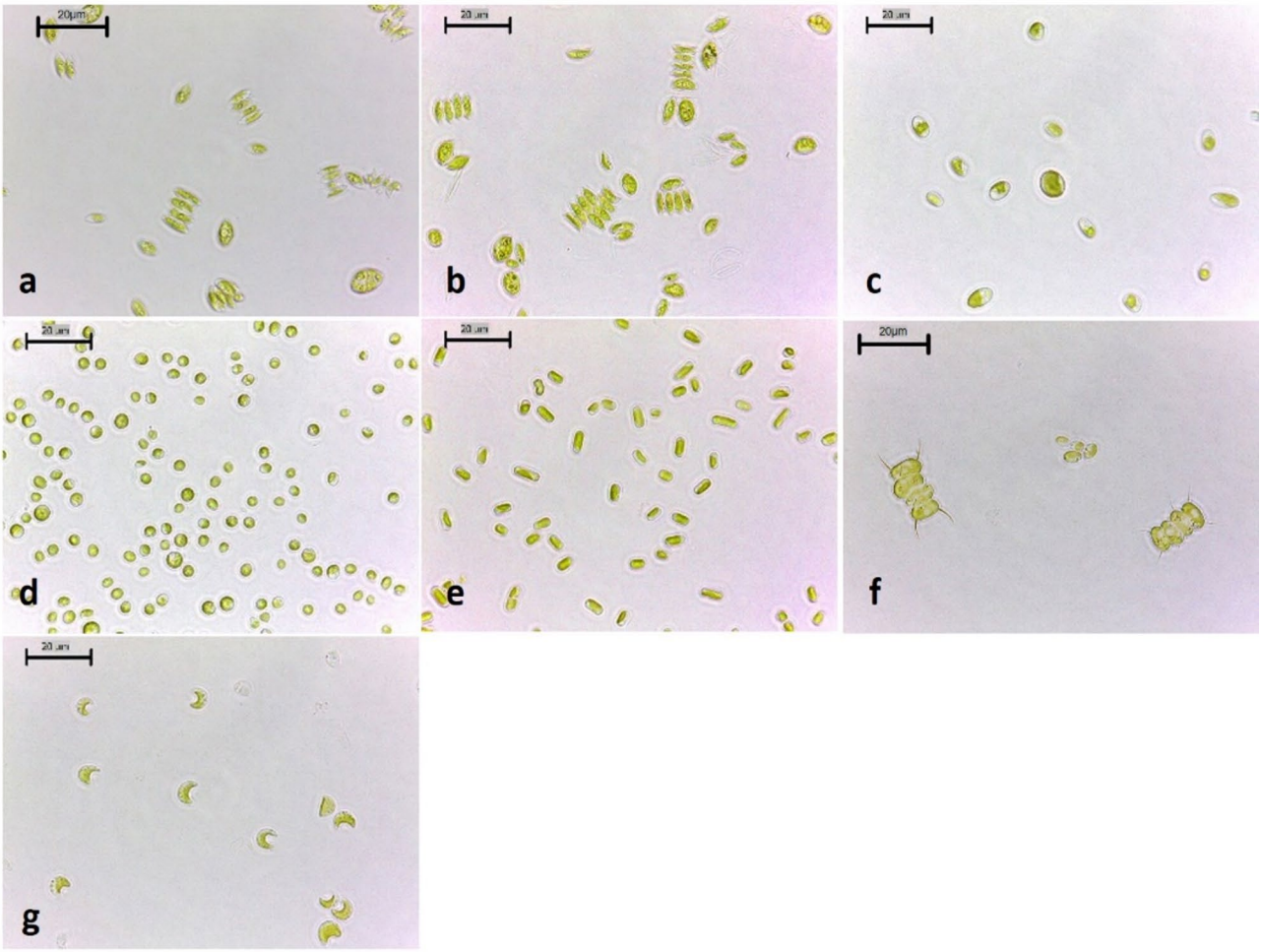


**Fig. 1**. Micrographs of (a) *Tetradesmus obliquus* FAM 27852, (b) *Tetradesmus obliquus* FAM 27855, (c) *Chloroidium saccharophilum* FAM 27962, (d) *Chlorella vulgaris* FAM 27965, (e) *Stichococcus* sp. FAM 27986, (f) *Desmodesmus* sp. FAM 28090, and (g) *Tetranephris brasiliensis* FAM 28097. Scale bars represent 20 µm.

in all strains, whereas oleic acid (C18:1*n*−9) was the most abundant MUFA, except for *C. saccharophilum* FAM 27962 biomass.

## Discussion

The microalgae commercial sector in Switzerland is still incipient. Unlike in other European algal-producing countries, the available information is fragmented, and the general scenario is not precisely known[3]. The development of a culture collection of native Swiss microalgae strains promotes not only the maintenance and documentation of national biodiversity, but also encourages microalgae commercial exploitation. Using a similar approach, Agroscope maintains an extensive collection of microorganisms involved in cheesemaking (mainly lactic acid and propionic acid bacteria) which are marketed by a limited company[15]. Moreover, a microalgae culture collection represents a source of potentially interesting biomolecules. It is known that using locally dominant microalgae strains is better for biotechnological exploitation, as these strains are well adapted to local conditions and do not pose ecological risk as invasive species[2]. Bao et al.[5] performed a similar study in Australia, isolated 40 local strains, and found that three strains had great potential for commercial exploitation in the nutraceutical, functional food, and animal feed sectors (i.e., *Scenedesmus* sp. GW63, *Desmodesmus* sp. UQL1_26, and *Monoraphidium convolutum* GW5). Likewise, after a comprehensive screening of 101 strains isolated in China, Zhang et al.[16] suggested that *Desmodesmus* sp. WC08 would be a good candidate for biodiesel production. The commercialization of the cyanobacterium *Aphanizomenon flos-aquae*, which occurs naturally in Upper Klamath Lake (Oregon, USA), is an example of exploitation based on fitness to local conditions[17]. The streamlined sampling and isolation process described in this study is an ongoing effort to sample the entire Swiss territory and, to the best of the authors' knowledge, is the first work of this kind in Switzerland.

The selection of a desirable strain for biotechnological exploitation must be done by considering the calculation of productivity, which determines the cultivation time needed to produce a certain amount of biomass or bioproduct of interest[18]. Ultimately, productivity depends on the growth rate and on the biomass concentration that a strain can achieve within a given timeframe (Fig. 2). It is also influenced by the physicochemical cultivation

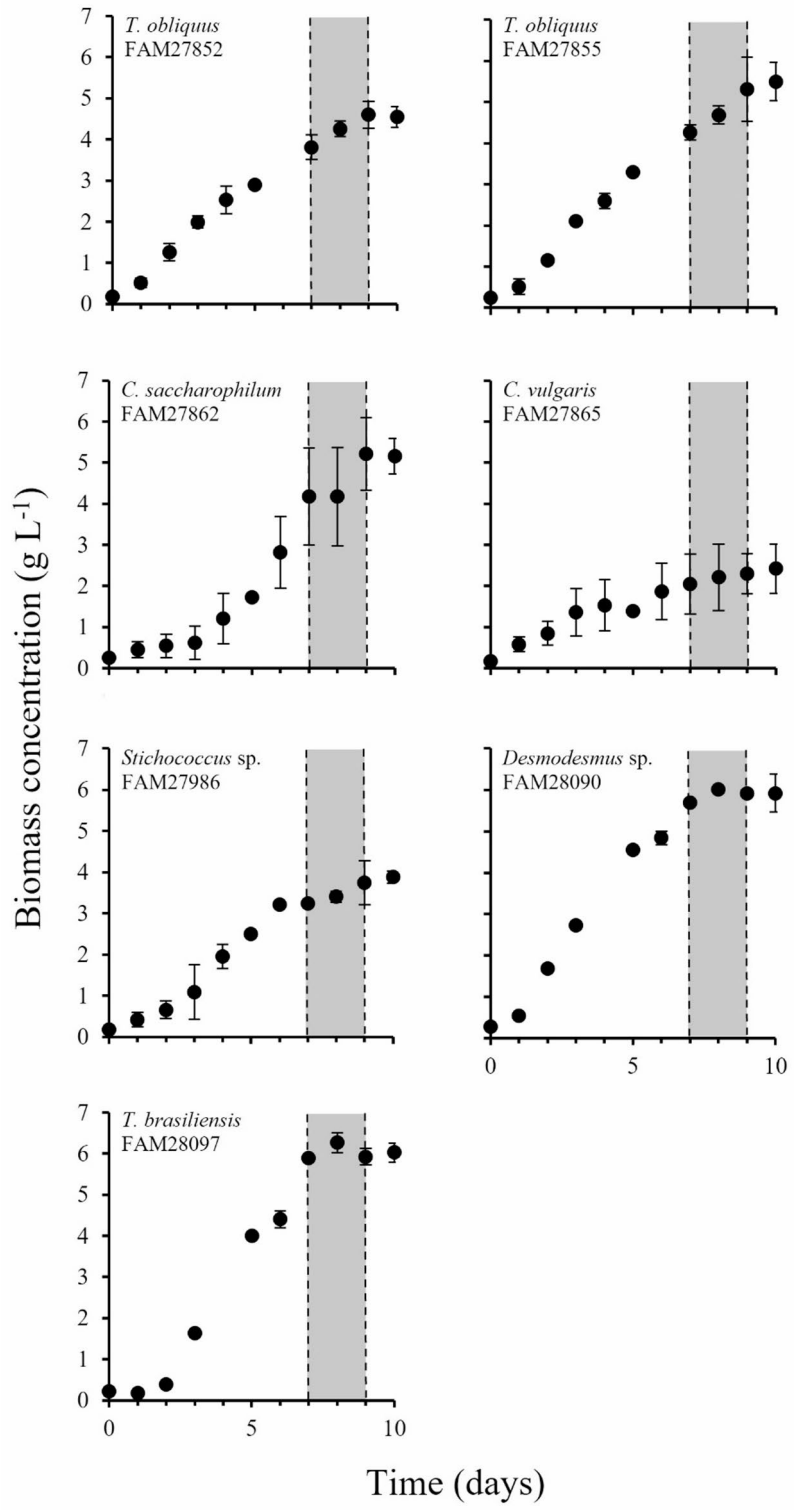


**Fig. 2**. Photoautotrophic growth curves of native Swiss microalgae strains grown in Doucha's growth medium at 22.5 °C, with 150 µmol photons $m^{-2}$ $s^{-1}$ in a 4% $CO_2$-enriched atmosphere. The gray areas represent the time interval in which cells were harvested for analyses (late exponential phase). Data points are means of at least three independent experiments, and error bars represent SD.

conditions. Using the cultivation conditions described in the present study, growth parameters were generally high when compared to those in the literature (Table 1). However, such a comparison of results is challenging because cultivation conditions vary widely in the literature. For example, *T. obliquus* presented photoautotrophic maximal biomass concentrations and productivities that varied from 0.56 g $L^{-1}$ to 20.14 g $L^{-1}$ and 0.06 g $L^{-1}$ $d^{-1}$

| Strain | Specific growth rate, μ ($d^{-1}$) | Max. biomass concentration ($g\ L^{-1}$) | Biomass productivity ($g\ L^{-1}\ d^{-1}$) |
|---|---|---|---|
| *T. obliquus* FAM27852 | $0.60 \pm 0.05^{b}$ | $4.66 \pm 0.33^{bc}$ | $0.50 \pm 0.04^{b}$ |
| *T. obliquus* FAM27855 | $0.62 \pm 0.04^{b}$ | $5.58 \pm 0.59^{ab}$ | $0.58 \pm 0.10^{abc}$ |
| *C. saccharophilum* FAM27962 | $0.52 \pm 0.05^{bc}$ | $5.45 \pm 0.67^{ab}$ | $0.56 \pm 0.09^{abc}$ |
| *C. vulgaris* FAM27965 | $0.41 \pm 0.01^{c}$ | $2.42 \pm 0.60^{d}$ | $0.24 \pm 0.06^{d}$ |
| *Stichococcus* sp. FAM27986 | $0.56 \pm 0.03^{b}$ | $3.97 \pm 0.32^{c}$ | $0.41 \pm 0.06^{cd}$ |
| *Desmodesmus* sp. FAM28090 | $0.77 \pm 0.02^{a}$ | $6.14 \pm 0.07^{a}$ | $0.66 \pm 0.08^{ab}$ |
| *T. brasiliensis* FAM28097 | $0.80 \pm 0.07^{a}$ | $6.27 \pm 0.25^{a}$ | $0.73 \pm 0.11^{a}$ |

**Table 1**. The specific growth rate, maximum biomass concentration, and biomass productivity of native Swiss microalgae strains grown in doucha's growth medium at 22.5 °C, with 150 μmol photons $m^{-2}\ s^{-1}$ in a 4% $CO_2$-enriched atmosphere. The data represent the mean ± SD of at least three independent experiments. Different superscript letters in the same column indicate significantly different values ($p < 0.05$).

| Strain | Proteins (%) | Lipids (%) | Carbohydrates (%) | Ashes (%) | Chlorophyll a + b ($mg\ g^{-1}$) | Carotenoids ($mg\ g^{-1}$) |
|---|---|---|---|---|---|---|
| *T. obliquus* FAM27852 | $45.15 \pm 0.34^{c}$ | $12.01 \pm 0.07^{d}$ | $15.27 \pm 1.44^{c}$ | $4.68 \pm 0.01^{c}$ | $25.30 \pm 0.34^{b}$ | $4.95 \pm 0.18^{b}$ |
| *T. obliquus* FAM27855 | $46.17 \pm 0.17^{c}$ | $14.29 \pm 0.29^{bc}$ | $19.08 \pm 1.50^{bc}$ | $4.86 \pm 0.02^{c}$ | $18.05 \pm 0.86^{c}$ | $3.04 \pm 0.18^{cd}$ |
| *C. saccharophilum* FAM27962 | $47.86 \pm 0.22^{b}$ | $14.12 \pm 0.44^{bc}$ | $19.66 \pm 1.30^{b}$ | $5.51 \pm 0.04^{b}$ | $32.08 \pm 0.49^{a}$ | $6.03 \pm 0.24^{a}$ |
| *C. vulgaris* FAM27965 | $36.93 \pm 1.01^{d}$ | $13.74 \pm 0.29^{c}$ | $20.24 \pm 2.35^{ab}$ | $4.13 \pm 0.05^{d}$ | $21.55 \pm 0.83^{c}$ | $3.84 \pm 0.36^{c}$ |
| *Stichococcus* sp. FAM27986 | $32.88 \pm 0.80^{e}$ | $14.72 \pm 0.00^{b}$ | $23.94 \pm 1.15^{a}$ | $3.17 \pm 0.13^{e}$ | $29.24 \pm 0.25^{a}$ | $5.61 \pm 0.22^{ab}$ |
| *Desmodesmus* sp. FAM28090 | $44.96 \pm 0.14^{c}$ | $9.69 \pm 0.05^{e}$ | $16.94 \pm 1.51^{bc}$ | $4.10 \pm 0.02^{d}$ | $10.21 \pm 2.73^{e}$ | $1.65 \pm 0.60^{e}$ |
| *T. brasiliensis* FAM28097 | $53.54 \pm 0.11^{a}$ | $18.08 \pm 0.00^{a}$ | $9.32 \pm 0.13^{d}$ | $8.19 \pm 0.06^{a}$ | $14.47 \pm 0.52^{d}$ | $2.53 \pm 0.22^{d}$ |

**Table 2**. The biochemical composition (in % DW) of native Swiss microalgae strains grown in doucha's growth medium at 22.5 °C, with 150 μmol photons $m^{-2}\ s^{-1}$ in a 4% $CO_2$-enriched atmosphere. The data represent the mean ± SD of at least three independent measurements. Different superscript letters in the same column indicate significantly different values ($p < 0.05$).

to 1.19 g $L^{-1}$ $d^{-1}$ depending on the cultivation system and strategy[19]. In conditions somewhat similar to those in this study, this microalga showed growth rates of 0.94 $d^{-1}$ – 1.19 $d^{-1}$, which are substantially higher than the ones presented here[20,21]. Nevertheless, strain-specific traits and minor cultivation differences can lead to rather diverse outcomes. Interestingly, the two *T. obliquus* strains investigated in this study had no significant differences in growth. As a general trend, specific growth rates found in this study were within the broad ranges found in the literature, like 0.42 $d^{-1}$–1.14 $d^{-1}$ for *Chloroidium* spp[22]., 0.29 $d^{-1}$–0.89 $d^{-1}$ for *C. vulgaris*[23,24], 0.25 $d^{-1}$–0.72 $d^{-1}$ for *Stichococcus bacillaris*[25,26], and 0.25 $d^{-1}$–0.68 $d^{-1}$ for *Desmodesmus communis*[27,28]. In contrast, the maximum biomass concentrations and productivities observed in this study were higher than those typically reported, which can be due to strain-specific characteristics and/or to the standardized growth conditions applied. However, it should be noted that the cultivation in this study was carried out in a highly controlled environment, in small-scale systems, and was not optimized for specific species. Therefore, although the results for bulk biomass production were promising, further optimization studies are necessary before the upscaling and biotechnological exploitation of the strains investigated. For example, the selection of 5-L bottles with a relatively large diameter as cultivation flasks favors self-shading, inhibiting light penetration throughout the entire culture. Hence, using flasks with a shorter diameter may lead to better results and must be part of the proposed optimization process.

Microalgal biomass and/or extracts have been successfully used for nutrient upcycling, wastewater treatment, food and feed production and/or supplementation, development of bioactive molecules, and other industrially relevant applications[29–32]. To select the ideal strain for a given application or vice-versa, the biochemical composition of the biomass is a crucial characteristic that must be assessed[33]. For example, a microalgal strain suitable for exploitation as an alternative source of protein for food or feed must have an appropriate protein

| Amino acid | *T. obliquus* FAM27852 | *T. obliquus* FAM27855 | *C. saccharophilum* FAM27962 | *C. vulgaris* FAM27965 | *Stichococcus sp.* FAM27986 | *Desmodesmus sp.* FAM28090 | *T. brasiliensis* FAM28097 |
|---|---|---|---|---|---|---|---|
| *Essential* | | | | | | | |
| Histidine | 1.94 ± 0.03 | 1.85 ± 0.03 | 1.89 ± 0.02 | 1.90 ± 0.03 | 1.94 ± 0.03 | 2.11 ± 0.01 | 1.90 ± 0.01 |
| Isoleucine | 4.05 ± 0.03 | 4.10 ± 0.01 | 4.01 ± 0.02 | 3.65 ± 0.10 | 4.10 ± 0.11 | 3.73 ± 0.03 | 3.87 ± 0.00 |
| Leucine | 8.94 ± 0.06 | 9.06 ± 0.02 | 8.29 ± 0.02 | 9.27 ± 0.30 | 9.03 ± 0.24 | 8.55 ± 0.05 | 9.09 ± 0.03 |
| Lysine | 5.85 ± 0.02 | 5.89 ± 0.02 | 6.34 ± 0.03 | 6.04 ± 0.16 | 6.07 ± 0.16 | 6.51 ± 0.00 | 5.88 ± 0.01 |
| Methionine | 2.12 ± 0.04 | 2.12 ± 0.02 | 1.47 ± 0.04 | 2.19 ± 0.01 | 1.78 ± 0.04 | 2.15 ± 0.05 | 2.23 ± 0.04 |
| Phenylalanine | 5.50 ± 0.03 | 5.60 ± 0.02 | 5.08 ± 0.04 | 5.44 ± 0.14 | 5.64 ± 0.14 | 5.06 ± 0.03 | 5.44 ± 0.06 |
| Threonine | 5.57 ± 0.07 | 5.31 ± 0.02 | 4.72 ± 0.03 | 4.46 ± 0.14 | 5.35 ± 0.16 | 5.57 ± 0.07 | 4.35 ± 0.01 |
| Tryptophan | 2.23 ± 0.02 | 2.29 ± 0.05 | 1.65 ± 0.04 | 2.15 ± 0.02 | 1.75 ± 0.01 | 2.09 ± 0.01 | 2.34 ± 0.00 |
| Valine | 5.83 ± 0.04 | 5.74 ± 0.02 | 5.07 ± 0.01 | 5.71 ± 0.20 | 5.79 ± 0.16 | 5.43 ± 0.00 | 5.29 ± 0.01 |
| *Non-essential* | | | | | | | |
| Alanine | 8.45 ± 0.06 | 8.55 ± 0.06 | 6.95 ± 0.02 | 8.38 ± 0.30 | 8.41 ± 0.22 | 7.91 ± 0.02 | 8.47 ± 0.04 |
| Arginine | 6.29 ± 0.03 | 7.29 ± 0.05 | 6.42 ± 0.04 | 7.68 ± 0.14 | 6.30 ± 0.17 | 6.63 ± 0.03 | 11.12 ± 0.01 |
| Asparagine | 8.92 ± 0.05 | 9.03 ± 0.05 | 9.74 ± 0.10 | 9.33 ± 0.26 | 9.58 ± 0.22 | 9.39 ± 0.00 | 8.62 ± 0.03 |
| Cysteine | 1.97 ± 0.02 | 1.74 ± 0.07 | 1.06 ± 0.00 | 1.54 ± 0.03 | 1.42 ± 0.02 | 1.79 ± 0.03 | 1.52 ± 0.04 |
| Glutamine | 10.92 ± 0.06 | 11.25 ± 0.02 | 18.16 ± 0.20 | 12.38 ± 0.42 | 11.60 ± 0.27 | 11.63 ± 0.02 | 10.75 ± 0.09 |
| Glycine | 7.41 ± 0.09 | 6.43 ± 0.03 | 5.84 ± 0.03 | 5.99 ± 0.20 | 6.43 ± 0.19 | 8.25 ± 0.24 | 6.69 ± 0.01 |
| Proline | 5.17 ± 0.08 | 5.06 ± 0.02 | 5.52 ± 0.02 | 5.54 ± 0.16 | 6.32 ± 0.18 | 5.20 ± 0.00 | 4.85 ± 0.03 |
| Serine | 4.37 ± 0.09 | 4.28 ± 0.05 | 4.16 ± 0.04 | 4.46 ± 0.10 | 4.54 ± 0.11 | 4.10 ± 0.07 | 3.78 ± 0.04 |
| Tyrosine | 4.49 ± 0.06 | 4.41 ± 0.03 | 3.63 ± 0.02 | 3.89 ± 0.08 | 3.95 ± 0.12 | 3.90 ± 0.08 | 3.82 ± 0.01 |

**Table 3**. The amino acid composition (in % of total proteins) of native Swiss microalgae strains grown in doucha's growth medium at 22.5 °C, with 150 µmol photons $m^{-2}$ $s^{-1}$ in a 4% $CO_2$-enriched atmosphere. The data represent the mean ± SD of at least three independent measurements.

| Fatty acid | *T. obliquus* FAM27852 | *T. obliquus* FAM27855 | *C. saccharophilum* FAM27962 | *C. vulgaris* FAM27965 | *Stichococcus sp.* FAM27986 | *Desmodesmus sp.* FAM28090 | *T. brasiliensis* FAM28097 |
|---|---|---|---|---|---|---|---|
| *SFAs* | | | | | | | |
| C14:0 | 0.22 ± 0.01 | 0.21 ± 0.00 | 0.40 ± 0.00 | 0.10 ± 0.04 | 0.89 ± 0.01 | 0.16 ± 0.01 | 0.36 ± 0.01 |
| C16:0 | 12.34 ± 0.17 | 12.94 ± 0.07 | 13.24 ± 0.20 | 13.73 ± 0.14 | 16.97 ± 0.01 | 12.93 ± 0.23 | 9.36 ± 0.09 |
| C18:0 | 0.25 ± 0.01 | 0.25 ± 0.01 | 0.15 ± 0.03 | 0.21 ± 0.00 | 0.55 ± 0.00 | 0.27 ± 0.01 | 0.17 ± 0.02 |
| Total SFAs | 13.53 ± 0.17 | 14.21 ± 0.09 | 14.39 ± 0.20 | 14.53 ± 0.14 | 19.84 ± 0.02 | 14.52 ± 0.08 | 10.39 ± 0.04 |
| *MUFAs* | | | | | | | |
| C16:1*n*−3 | 3.86 ± 0.04 | 3.57 ± 0.02 | 2.61 ± 0.02 | 3.97 ± 0.03 | 2.10 ± 0.04 | 4.96 ± 0.02 | 7.57 ± 0.13 |
| C17:1*n*−10 | 4.21 ± 0.01 | 3.96 ± 0.06 | 1.43 ± 0.03 | 4.27 ± 0.01 | 5.89 ± 0.04 | 5.99 ± 0.06 | 0.32 ± 0.01 |
| C18:1*n*−9 | 8.50 ± 0.01 | 11.66 ± 0.00 | 1.69 ± 0.06 | 5.88 ± 0.06 | 25.16 ± 0.23 | 12.98 ± 0.30 | 16.80 ± 0.40 |
| C18:1*n*−11 | 1.32 ± 0.02 | 1.60 ± 0.01 | 0.47 ± 0.01 | 1.58 ± 0.01 | 1.54 ± 0.01 | 1.42 ± 0.01 | 1.23 ± 0.06 |
| Total MUFAs | 18.44 ± 0.01 | 21.42 ± 0.07 | 7.00 ± 0.17 | 16.02 ± 0.14 | 35.57 ± 0.35 | 26.17 ± 0.45 | 28.07 ± 0.62 |
| *PUFAs* | | | | | | | |
| C18:2*n*−9 | 18.56 ± 0.09 | 19.07 ± 0.07 | 43.74 ± 1.01 | 27.41 ± 0.21 | 11.44 ± 0.16 | 12.23 ± 0.30 | 9.64 ± 0.22 |
| C18:3*n*−6 | 1.57 ± 0.00 | 2.72 ± 0.01 | n.d. | n.d. | 0.14 ± 0.00 | 1.98 ± 0.09 | 0.06 ± 0.01 |
| C18:3*n*−9 | 1.49 ± 0.09 | 1.21 ± 0.01 | 9.63 ± 0.18 | 14.08 ± 0.07 | 2.23 ± 0.01 | 16.38 ± 0.30 | 11.33 ± 0.22 |
| C18:4*n*−3 | 2.17 ± 0.01 | 2.31 ± 0.01 | n.d. | n.d. | 0.11 ± 0.01 | 2.92 ± 0.06 | 2.78 ± 0.07 |
| Total PUFAs | 37.84 ± 0.11 | 36.20 ± 0.08 | 53.49 ± 1.28 | 41.54 ± 0.35 | 35.29 ± 0.17 | 33.51 ± 0.75 | 23.83 ± 0.58 |
| Total ω−3 | 17.73 ± 0.11 | 14.42 ± 0.20 | 9.63 ± 0.18 | 14.08 ± 0.07 | 22.71 ± 0.12 | 19.31 ± 0.38 | 14.13 ± 0.27 |
| Total ω−6 | 20.12 ± 0.22 | 21.78 ± 0.51 | 43.83 ± 1.01 | 27.46 ± 0.14 | 12.46 ± 0.12 | 14.202 ± 0.38 | 9.73 ± 0.27 |
| Total FAs (%DW) | 8.68 ± 0.04 | 10.20 ± 0.04 | 10.46 ± 0.22 | 10.38 ± 0.73 | 12.64 ± 0.15 | 9.40 ± 0.17 | 15.93 ± 0.22 |

**Table 4**. The fatty acid composition (in % of total fatty acids) and total fatty acid content (% DW) of native Swiss microalgae strains grown in doucha's growth medium at 22.5 °C, with 150 µmol photons $m^{-2}$ $s^{-1}$ in a 4% $CO_2$-enriched atmosphere. The data represent the mean ± SD of at least three independent measurements. Non detected fatty acids are marked with "n.d.".

content in its biomass. Similarly, for biodiesel production from microalgae, a strain must have the desirable amounts of lipids. Briefly, that is how the biotechnological potential of microalgae is assessed. In general, ash and protein concentrations found in this study differed slightly from those in the literature. It has been indicated that proteins in green algae are generally 10–40% of the dry biomass, and ashes are 6–14%[5,34]; these values are below and above the ones found here, respectively (Table 2). However, such differences must be interpreted with caution, since, in contrast to the present study, the available data comes predominantly from marine microalgae, which strongly affects cell composition[35]. Not only seawater, but changes in the chemical composition of the growth medium, such as nutrient limitation and/or starvation, have traditionally been used to induce the accumulation of certain bioproducts. For example, when deprived of nitrogen, eukaryotic green microalgae tend to accumulate storage compounds, such as neutral lipids or starch, depending on the strain[36].

Many interests have been directed to the use of (macro- and) microalgae as food, feed, or supplement over the last few years[37]. For example, in Switzerland there is a strong effort to minimize soy importation as a protein source for animal feed, and protein-rich microalgae are potential candidates to address this issue in a sustainable manner. Not only is the concentration of proteins in the microalgal biomass important for its nutritional assessment, but so is its amino acid composition, since not all amino acids in diet are equally important[38]. The amino acid profiles of all strains investigated in this study were highly similar (Table 3), as already noted by other authors who have studied other microalgae[39,40]. Interestingly, the amino acid profile of microalgae does not appear to be affected by case-dependent parameters, such as irradiance, growth phase, harvest regime, or nutrient supply[39]. In comparison to soy protein[41], the amino acid composition of the investigated microalgae revealed higher concentrations of the non-essential amino acids alanine and glycine as well as of the essential amino acids leucine, methionine, phenylalanine, threonine, and tryptophan. When compared to whey protein[41], alanine, arginine, glycine, and tyrosine were the non-essential amino acids that were more abundant in microalgae, whereas methionine, phenylalanine, and tryptophan were the essential ones. These comparisons confirm the great potential of native Swiss microalgae as alternative protein sources for food and feed purposes. In pigs' diets, for example, the amino acids lysine, methionine, cysteine, threonine, and tryptophan are of particular importance[42]. Their concentrations in microalgae biomass are all equal or superior of those found in soybean meal (feedbase.ch). It is noteworthy that the use of microalgae or microalgae extracts in the food sector in Europe requires prior authorization of the European Food Safety Authority (EFSA). From the isolated strains reported in this study, only *C. vulgaris* FAM 27965 has the authorization to be used as a food or food ingredient. In Switzerland, there are no restrictions for the use of microalgae in animal feed, thereby reinforcing the great potential of the Swiss microalgal biotechnology sector in this area.

Total lipids and fatty acid composition are important parameters in the selection of potential applications for microalgae, such as in the food, feed, and biofuel sectors. While oil productivity can be calculated based on total lipids, the fatty acid profile computes the amount of essential PUFAs in the diet or the properties of biodiesel obtained via transesterification of fatty acids. The microalgal strains investigated in this study had a fairly similar proportion of saponifiable lipids in their total lipid contents (70–80%), except for *Desmodesmus* sp. FAM 28090 with virtually all its lipids containing fatty acids (97%) (Tables 2 and 4). The total fatty acids content in their dry biomass (8.68–15.93%) was higher than that found in other microalgae traditionally used in the food and aquaculture sectors, such as *Limnospira platensis*, *Chaetoceros muelleri*, *Isochrysis* sp., *Pavlova salina*, and *Botryococcus braunii*[43,44]. Moreover, the proportion of MUFAs and PUFAs in total fatty acids observed here were similar to commercial samples of *Limnospira*, *Chlorella*, *Nannochloropsis*, and *Phaeodactylum*, although the SFAs in the commercial samples were higher[39]. It should be noted that the values reported in the literature are widely distributed, and any comparison must consider the differences in cultivation conditions among studies. Unlike amino acids, the composition of fatty acids is known to be affected by cultivation conditions such as growth phase, nutrient availability, temperature, and $CO_2$ supply[39]. Therefore, differences within genera or among strains of the same species must be interpreted with caution.

An application-directed approach based on the fatty acid profiles indicates that the PUFA-rich oil (23.83–53.49% of total fatty acids) found in the microalgae strains isolated in Switzerland are not suitable for biodiesel production. The high degree of unsaturation of fatty acids is known to deteriorate biodiesel quality after transesterification[45]. In contrast, PUFAs are greatly appreciated in the food and feed sectors, as some of them are not naturally produced by animals and, therefore, essential in diets. For example, linoleic (18:2) and linolenic (18:3) acids are precursors of longer-chain (20 C and 22 C) *n*−3 and *n*−6 fatty acids, which have anti-inflammatory activity and reduce the risk of cancer and cardiovascular diseases in humans[46]. In animals, an increase in dietary docosahexaenoic acid (22:6*n*−3), synthesized from linolenic acid, has been linked to positive results in the growth, fertility, immunity, and bone strength of pigs and poultry[47]. Those fatty acids were consistently found in the microalgae strains investigated here (Table 4). When microalgae were fed to cows or laying hens, the milk and eggs were enriched with long-chain PUFAs[48,49]. Microalgae PUFAs have also been associated with the reduction of methane production in ruminants[50].

It is possible to obtain a glimpse of the potential applications of microalgae by analyzing their growth parameters and biochemical composition, as done in this study. However, some conclusions are not straightforward. For example, microalgal bioactive polysaccharides have been receiving attention recently, but very little is known about their structure and synthesis[51]. A few aspects regarding the production and release of microalgal extracellular polysaccharides appear to be related to the operational conditions of biomass production and are highly case-specific[52]. Therefore, in this study, carbohydrates were not investigated beyond their total content in microalgae biomass (9.32–23.94%), which are similar or lower than that normally found in the literature[51].

Pigments are also valuable molecules for biotechnological applications in the food, feed, cosmetic, and pharmaceutical sectors. Due to the presence of conjugated double bonds in their chemical structure, pigments usually have antioxidant activity, which carotenoids are particularly known for. However, carotenogenesis is usually induced under stress conditions such as high light, nutrient starvation, and salt stress[53]. Thus, the

standardized growth conditions used here were not suitable for addressing the potential of pigments for commercial applications. These topics should be investigated in future research.

## Conclusion

Switzerland's first microalgae culture collection (AlgoScope) is a source of potentially interesting strains, biomolecules, and biodiversity that could strengthen the national biotechnology sector. Sampling, isolation, and characterization efforts will continue with the goal of adequately sampling the entire Swiss territory. In this study, two strains stood out for their interesting biochemical composition under the standardized growth conditions described herein: (i) *C. saccharophilum* FAM 27962 with high PUFA content in total fatty acids and high protein content; and (ii) *T. brasiliensis* FAM 28097 with the highest protein content observed in this study. Based on the approach implemented here, these strains are good candidates for independent or mixed use as feed material. However, scale-up feasibility analysis and animal acceptance tests need to be conducted before actual implementation.

## Materials and methods

### Microalgae sampling and isolation

Microalgae strains were isolated from samples collected from a wide variety of Swiss environments (see Supplementary information), in locations with favorable conditions for algal growth, such as light, humidity, and nutrient availability. Ideally, the first purification steps were carried out immediately after sampling. In nonoptimal occasions, samples were stored at low temperature (4–8 °C) before purification and isolation. The first purification step involved large-pore filtration (8 µm cellulose nitrate membrane filters; Sartorius) to separate individual microalgae from sample residues and debris. The permeate was then subjected to a second filtration using a smaller pore filter (1.2 µm cellulose nitrate membrane filters; Sartorius) where smaller contaminants were removed in the permeate and the retentate was maintained. This step was repeated two to four times and the retentates were rinsed with sterile distilled water between each filtration. The retentate from the last filtration was finally collected and applied to standard agar plates containing Doucha's growth medium[54] with 3.5% agar. Inoculums were applied to the plates either using a seeding loop or by spraying the retentate diluted in water. Plates were incubated under standard conditions (see Sect. 2.3) and monitored regularly. Individual colonies were reapplied to new agar plates as many times as necessary until it appeared pure on the plate and under the optical microscope. For the microscopic monitoring of plates, sample colonies were resuspended in liquid medium and examined using optical microscopy (Leica ICC50 HD, Leica). The successfully purified strains were characterized as described below and maintained in AlgoScope, the microalgae culture collection of Agroscope (Switzerland). All relevant information regarding the strains, including the micrographs, were documented in an internal database[55].

### Identification through DNA sequencing

A 100-mg sample of plated microalgal culture was collected and placed into 550 µL of CD1 buffer (DNeasy Plant Pro Kit, ref. 69204). Samples were processed using a TissueLyser (TissueLyser II Qiagen, ref. 1218130627E) for 3 minutes at 24 Hz. The homogenized 550 µL was transferred to a 2 mL collection tube and subjected to DNA extraction using a QIAcube system (QIAcube Connect Priority Blue 279, ref. 9002842). The eluted DNA was recovered in 100 µL of EB buffer (Qiagen). Amplification was performed on 5 µL of DNA in a reaction mixture containing 2.5 µL of 10x Buffer (Qiagen), 2.25 µL of 25 mM $MgCl_2$ (Qiagen), 1.25 µL each of ITS4 (5'-TCCTCCGCTTATTGATATGC-3'; 10 mM stock) and ITS5 (5'-GGAAGTAAAAGTCGTAACAAGG-3'; 10 mM stock) primers, 1 µL of dNTPs, 1 µL of betaine, 0.125 µL of Taq DNA polymerase (Qiagen), and 10.625 µL of Milli-Q water. PCR was conducted using a thermocycler (ALG4/5) under the following conditions: an initial denaturation step of 3 min at 96 °C, followed by 1 min at 42 °C and 1 min at 72 °C for 4 cycles. This was followed by 25 cycles of 1 min at 93 °C, 90 s at 51 °C, and 1 min at 72 °C, with a final extension step of 5 min at 72 °C[56]. PCR products were visualized on a 1.8% agarose gel via electrophoresis. Then, strain identification was performed based on the PCR products in accordance with White et al.[57]. Sequencing was performed using Genesupport SA (Fasteris Sanger Services, CH-1228 Plan-les-Ouates). The resulting sequences were compared against the GenBank BLAST® database (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to identify species.

### Standard cultivation conditions and growth monitoring

The isolated strains were cultivated in standard conditions to maximize biomass production. Cultivation conditions were selected after the preliminary optimization of physicochemical cultivation parameters for multiple strains. Microalgae were cultivated in 5-L glass bottles with membrane venting caps (Duran®) containing 4 L of Doucha's growth medium (pH 7.0) carrying: 0.55 g $CO(NH_2)_2$, 0.12 g $KH_2PO_4$, 0.10 g $MgSO_4$ $7H_2O$, 0.02 g $Na_2EDTA$ Fe, 0.09 g $CaCl_2$ $2H_2O$, and 2 mL microelements solution per liter[54]. The volume of the growth medium was visually monitored and, when necessary, adjusted by adding sterile water to minimize evaporation effects. Bottles were inoculated with 0.2 g $L^{-1}$ of dry biomass and maintained in an incubator (Multitron, Infors-HT) at 22.5 °C with 115 rpm orbital agitation and continuous illumination of 150 µmol photons $m^{-2}$ $s^{-1}$ in a 4% $CO_2$-enriched atmosphere. Growth was followed daily using a spectrophotometer (DR 3900, Hach). The absorbance at 750 nm was converted to dry weight based on a calibration curve obtained after serial dilution of microalgae cultures. For this purpose, the samples of cell suspension were filtered through pre-weighted glass fiber filters (Whatman GF/B, Cytiva) and oven-dried at 105 °C until constant weight was reached. The specific growth rates (µ) were estimated from the linear coefficient of the equation that describes the exponential portion of the logarithmic growth curve of each strain. The biomass productivity (g $L^{-1}$ $d^{-1}$) was calculated considering only the exponential growth phase, by dividing the biomass concentration (g $L^{-1}$) at a given time by the time in

days elapsed to achieve it. At the end of the exponential growth phase, cells were harvested via centrifugation (Bio Stratos, Thermo Scientific), lyophilized, and stored at −40 °C until further analysis.

## Macromolecular composition

The biochemical characterization of strains involved the measurement of ashes, lipids, proteins, pigments, and carbohydrates in the dry biomass. Amino acids and fatty acids profiles were also determined. Protein content was determined as the sum of amino acids estimated using ultra-high liquid chromatography (see section Amino acids profiles). For the determination of the total amount of carbohydrates, algal biomass was first subjected to inorganic acid hydrolysis. Briefly, dry algal samples were suspended in 72% (w/w) sulfuric acid and incubated for 1 h at 30 °C, followed by 1 h at 121 °C in 4% (w/w) sulfuric acid in an autoclave. The concentration of monosaccharides in the resulting hydrolysate was determined colorimetrically at 620 nm based on the bonding of free aldehyde groups to 3-methyl-2-benzothiazolinone hydrazone (MBTH), as described by Van Wychen and Laurens[58].

For the determination of total lipids, samples were subjected to a different acid hydrolysis (ISO 6492:1999). After being boiled in 10% (w/w) hydrochloric acid for 1 h, the hydrolysate was filtered, neutralized, and dried at room temperature. Crude lipids were extracted from the dry hydrolysate under pressure with petroleum ether (distillation range 40–60 °C). The residue was oven-dried under reduced pressure, and the proportion of total lipids was determined gravimetrically. The ashes content was determined after incinerating the samples at 550 °C until constant weight was reached (ISO 5984:2002). Further, chlorophylls and carotenoids were extracted overnight at −20 °C with 100% methanol after samples were disrupted by bead milling with 0.2 mm glass beads (Bead Ruptor Elite, Omni International, USA). Pigment concentration in methanolic extracts was determined spectrophotometrically (470 nm, 653 nm, and 666 nm) in the following manner[59]:

Chlorophyll *a* ($\mu g\ mL^{-1}$) = $15.65 \times A_{666} - 7.34 \times A_{653}$.

Chlorophyll *b* ($\mu g\ mL^{-1}$) = $27.05 \times A_{653} - 11.21 \times A_{666}$.

Carotenoids $_{x+c}$ ($\mu g\ mL^{-1}$) = $(1{,}000 \times A_{470} - 2.86 \times Chl_a - 129.2 \times Chl_b)/245$.

where $A_{666}$, $A_{653}$, and $A_{470}$ are the absorbances at 666, 653, and 470 nm, respectively; $Chl_a$ and $Chl_b$ are the contents, in $\mu g\ mL^{-1}$, of chlorophyll *a* and chlorophyll *b*, respectively.

*Amino acids profiles*

To determine the amino acids profiles, samples were first incubated in 6 M hydrochloric acid for 15 h at 110 °C. The resulting solutions were neutralized and derivatized with an AccQ-Tag Ultra reagent (Waters, Switzerland). The amino acids profiles were determined using ultra-high performance liquid chromatography (2.1 mm × 100 mm, 1.7 µm; Acquity UPLC BEH C18, Waters) coupled to a UV detector (Ultimate 3000 RS, Thermo Scientific, Switzerland). Samples were incubated at 110 °C for 20 h in a 6 M hydrochloric acid solution containing 16 $\mu g\ mL^{-1}$ 1-methyl-tryptophan (internal standard) and 40 $mg\ mL^{-1}$ starch, and then gassed with $N_2$ for the determination of tryptophan. After cooling to room temperature, hydrolysates were neutralized, filtered, and measured by ultra-high liquid chromatography (2.1 mm × 150 mm, 1.7 µm; Acquity UPLC BEH C18, Waters). The amounts were normalized with a solution with known concentration of the internal standard.

*Fatty acids profiles*

The fatty acids profiles were determined by transmethylation/esterification, in accordance with Kragten et al.[60]. Briefly, samples were placed in a polytetrafluoroethylene tube with an internal standard solution (C19) and 5% hydrochloric acid in methanol. After three hours, the reaction mix was neutralized and purified by solid-phase extraction. The identification of fatty acids was carried out by gas chromatography coupled with a flame ionization detector (Agilent 6850, Agilent Technologies, Switzerland). Each fatty acid methyl ester was quantified using an internal standard C19.

## Statistical analyses

The data in this study are reported as mean ± standard deviation (SD) of at least three biologically independent replicates ($n \geq 3$). When necessary (i.e., amino acids and fatty acids profiles), biomasses from each biological replicate were pooled together in equal parts before analysis. The different values observed among strains were compared in GraphPad Prism 10.2.3 using one-way analysis of variance (ANOVA), followed by Tukey's honest significant difference test to determine whether there were significant differences among each of the investigated strains. All statistical analyses used a confidence level of 95% ($\alpha = 0.05$).

## Data availability

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

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Conceptualization: D.K. and A.B.B. Investigation: D.K., R.C., M.B.V., S.D., N.L., A.B.B. and K.G. Data Curation: D.K., S.D., N.L., and K.G. Formal Analysis: D.K., R.C., M.B.V., S.D., N.L., K.G., and A.B.B. Supervision: F.W. and A.B.B. Resources: F.W. and A.B.B. Writing - Original Draft: D.K. Writing - Review & Editing: All authors.

## Declarations

## Competing interests

The authors declare no competing interests.

## Additional information

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