J Ind Microbiol Biotechnol (2012) 39:1357–1365
DOI 10.1007/s10295-012-1133-x

ENVIRONMENTAL MICROBIOLOGY

Growth of *Dunaliella tertiolecta* and associated bacteria in photobioreactors

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Received: 2 January 2012 / Accepted: 16 April 2012 / Published online: 11 May 2012

Abstract The aim of this study was to test three flat-plate photobioreactor configurations for cultivation of marine green alga *Dunaliella tertiolecta* under non-axenic growth conditions and to characterize and quantify the associated bacteria. The photobioreactor cultivations were conducted using tap water-based media. Static mixers intended to enhance mixing and light utilization did not generally increase algal growth at the low light intensities used. The maximum biomass concentration (measured as volatile suspended solids) and maximum specific growth rate achieved in the flat plate with no mixer were 2.9 g l^{-1} and 1.3 day^{-1}, respectively. Based on quantitative polymerase chain reaction, bacterial growth followed the growth of *D. tertiolecta*. Based on 16S rDNA amplification and denaturing gradient gel electrophoresis profiling, heterotrophic bacteria in the *D. tertiolecta* cultures mainly originated from the non-axenic algal inocula, and tap water heterotrophs were not enriched in high chloride media (3 % salinity). Bacterial communities were relatively stable and reproducible in all flat-plate cultivations and were dominated by Gammaproteobacteria, Flavobacteria, and Alphaproteobacteria.

Keywords Microalgae · *Dunaliella tertiolecta* · Photobioreactor · Heterotrophic bacteria · Biomass yields

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Introduction

Biomass-based fuels are considered as sustainable alternatives to fossil fuels. Currently major share of biofuels and other forms of bioenergy are produced from terrestrial plants [34]. Microalgae may prove a supplementary energy source to terrestrial crops. Compared to plant harvest, microalgae have higher photosynthetic efficiencies, higher yields and growth rates, and fewer requirements for land-based cultivation space and they may be cultivated in saline waters and in arid land areas [28, 34].

Microalgal cultures can be grown in open pond systems or closed photobioreactors, as reviewed recently by Chisti [4], Schenk et al. [34] and Wiley et al. [40]. Open systems can be natural basins, or more often artificial ponds or containers. Photobioreactors can be of various shapes and sizes and include tubular photobioreactors [3, 10], flat-plate photobioreactors [6], column photobioreactors [14] and stirred tank reactors [8]. Compared to open systems, photobioreactors have reduced risk of contamination, more controlled cultivation conditions, improved control of strong turbulent flow, reduced CO_2 losses, prevention of water evaporation, increased volumetric yields, improved harvesting efficiency and increased illuminated area to volume ratio [3, 27]. On the other hand, photobioreactors have higher capital and operational costs, need cooling systems to prevent over-heating, are more complex and require trained personnel for proper operation [29].

In nature, algal growth is always associated with growth of other organisms and foremost bacteria [5, 30, 36]. Sterilization of the culture media and aseptic culture systems in large-scale microalgal biomass production are not economically or practically feasible for low cost sustainable energy. Thus, the characterization of heterotrophic associations in algal culture systems is an important step in

assessing microalgal technology for biofuel production, as heterotrophs contribute to total microbial biomass but may compete for available nutrients. In addition, bacteria can utilize compounds excreted by algae [39], increase solubility of nutrients and trace elements and make them more bioavailable for the microalgae [21], and help reduce dissolved O_2 saturation [24]. Analysis of the diversity of non-photosynthetic microorganisms can also provide useful information on the public health aspects by proving or excluding the presence of human, animal or plant pathogens in algal mass cultures.

The quantity and species composition of associated bacteria vary greatly between different algal species [9, 35]. Schäfer et al. [35] detected unique bacterial assemblages for marine diatoms *Dytilum brightwellii*, *Thalassiosira weissflogii*, *Asterionella glacialis*, *Chaetoceros socialis*, *Leptocylindrus danicus* and *Coscinodiscus* sp. grown in seawater based medium at 15 °C under illumination of 80–170 μmol photons m^{-2} s^{-1}, and all these algae coexisted with bacteria belonging to Cytophaga-Flavobacterium-Bacteroides (CFB) phylum and Alphaproteobacteria. Bacterial communities in cultures of diatoms and dinoflagellates *Guinardia delicatula*, *Pseudonitzschia pungens*, *Thalassiosira rotula*, *Skeletonema costatum*, *Ceratium horridum*, and *Akashiwo sanguine* originating from the North Sea were dominated by Alphaproteobacteria, Gammaproteobacteria and members of the Flavobacteria-Sphingobacteria group [33]. Rooney-Varga et al. [32] reported that both attached and free-living bacteria (collected with 5- and 0.22-μm pore size filters, respectively) in near-surface seawater samples with dinoflagellates and diatoms were dominated by members of the *Roseobacter* and *Cytophaga* groups.

Marine microalga *Dunaliella tertiolecta* is commercially important as it can contain more than 60 w-% lipids under certain growth conditions [38]. It is, therefore, a promising feedstock for production of biodiesel and renewable diesel. *Dunaliella* spp. have been successfully grown in open cultivation systems [29]. For this work, different flat-plate photobioreactor configurations were tested for cultivation of *D. tertiolecta* in tap water-based mineral salts medium. The source culture of *D. tertiolecta* was non-axenic, and in addition the tap water contained heterotrophs as reported in a previous study [22]. The quantity and quality of eukaryotes and bacteria in the non-axenic microalgal cultures were analyzed using culture-independent molecular tools based on denaturing gradient gel electrophoresis (PCR-DGGE) and quantitative polymerase chain reaction (qPCR). This work extends a previous study that examined growth of the fresh water microalga *Chlorella vulgaris* and associated heterotrophic bacteria [22]. It was hypothesized that high salinity of *D. tertiolecta* growth medium (3 %) compared to that of *C. vulgaris* (<0.05 %) and that the lack of rigid cell wall would lead to different composition and quantity of bacteria associated with microalgal cultures. Analytical procedures were similar to those used in our previous study [22] in order to provide comparable bacterial community data for both microalgal cultures.

Materials and methods

Test organisms and culture media

Dunaliella tertiolecta (SAG 13.86) was obtained as a non-axenic culture from the Culture Collection of Algae (SAG), University of Göttingen, Germany. Stock cultures were grown in modified NORO medium [38]. The culture was maintained in shake flasks at 22 ± 2 °C at 120 rpm under continuous 20 μmol photons m^{-2} s^{-1} illumination (Sylvania GRO-LUX F36 W/GRO-T8 fluorescent lamps). Stock cultures of *D. tertiolecta* contained heterotrophic bacteria and were transferred into sterile media at 2–4 week intervals.

Photobioreactors

Dunaliella tertiolecta was grown in tap water-based modified NORO medium in three different flat-plate photobioreactors; a flat-plate reactor with no mixer (designated as NM), a flat-plate reactor with a plain mixer (PM) and a flat-plate reactor with a complex mixer (CM). The flat-plate reactors were thin rectangular glass vessels with light path of 5 cm, illuminated area of 29.7 dm^2, total volume of 7.4 dm^3 and effective volume of 7.0 dm^3. The PM and CM also contained a removable mixing element parallel to the illuminated surfaces to enhance the mixing. The plain mixer was a smooth sheet, whereas the complex mixer was a sheet with baffles towards the aerated (upflow) side of the mixer. The design and configuration were identical to the photobioreactors previously described in [22].

The flat plates were continuously illuminated with four fluorescent lamps (Osram L 18W/965 biolux, two on each side) with light intensity of 50 μmol photons m^{-2} s^{-1}. Air/CO_2 mixture with 98 % air and 2 % CO_2 was introduced from the bottom of the reactors at 1.53 dm^3 min^{-1}. The air flows were controlled with multitube flow meters (Kytola Instruments E4K-LK04) and CO_2 flows with single tube flow meters (Aalborg P11A2-BAOA). The photobioreactors were inoculated with stock cultures previously grown in 1-l Erlenmeyer flasks on an orbital shaker (145 rpm) under three fluorescent lamps (Osram L 18W/965 biolux) for 2 weeks. The inoculum volumes per working volume of the reactor were 5.3 % (vol/vol). The reactors were acid

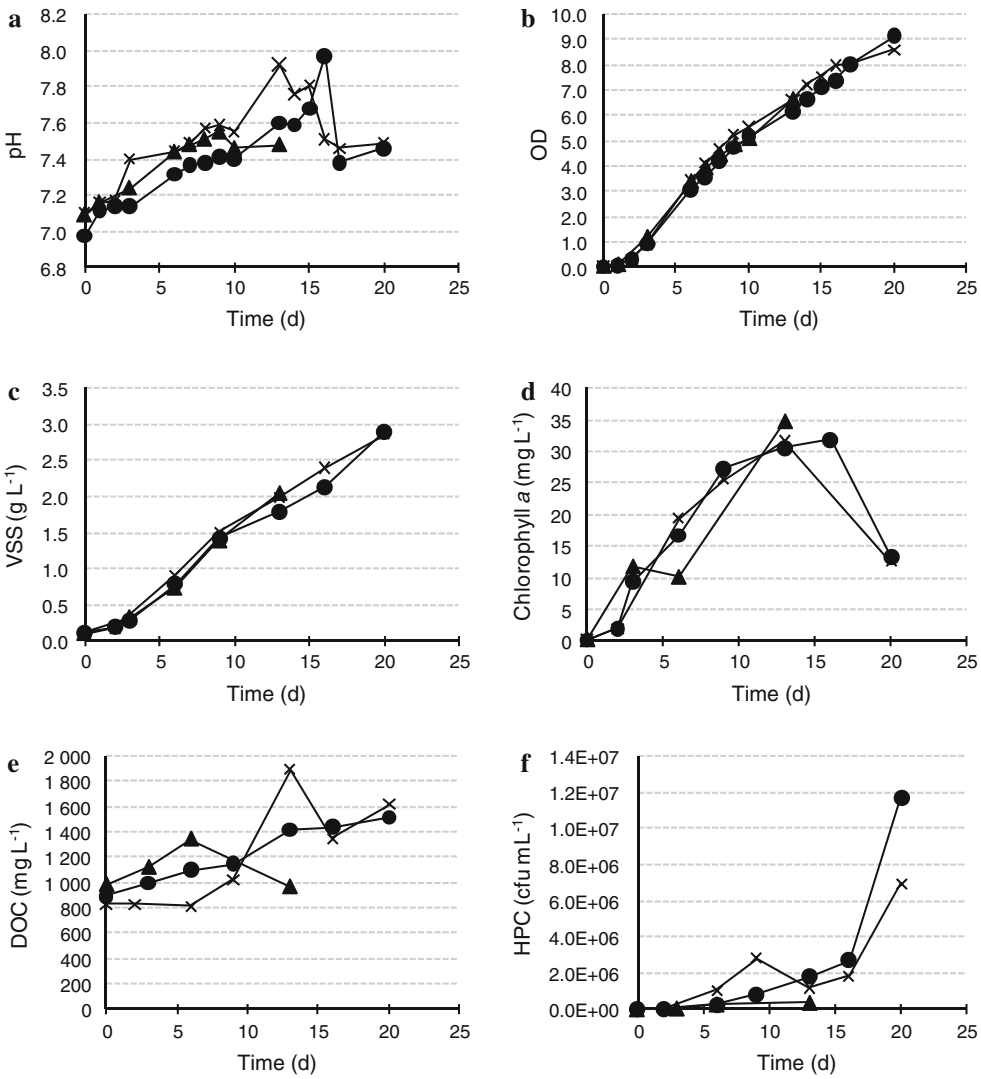

Fig. 1 Culture pH (**a**), optical density (OD) (**b**), volatile suspended solids (VSS) (**c**), chlorophyll *a* (**d**), dissolved organic carbon (**e**), and heterotrophic plate counts (HPC) (**f**) in *circle* the flat plate with no mixer, *triangle* the flat plate with the plain mixer, and *multiplication sign* the flat plate with the complex mixer

washed (1 M HCl) and rinsed with distilled water between the batch cultivations. The starting points of volatile suspended solids (VSS) were 0.12, 0.12, and 0.11 g l^{-1} and starting points of chlorophyll *a* were 0.21, 0.19, and 0.28 mg l^{-1} for NM, PM and CM, respectively (Fig. 1c, d). *Dunaliella tertiolecta* was batch cultivated in NM and CM reactors for 20 days, but due to a technical failure the PM cultivation was stopped on day 13.

Analytical methods

Optical density (OD) at 680 nm, temperature, pH, dissolved oxygen (DO), VSS, total suspended solids (TSS), chlorophyll *a*, dissolved organic carbon (DOC) and intensity of light were analyzed as previously described [22]. For heterotrophic plate counts (HPC), samples were diluted with 3 % NaCl solutions and 0.1-ml aliquots were spread plated on R2A-agar plates. The HPC plates were incubated at 26 °C for 7 days.

Microbial community analyses

Duplicate samples of 8–20 ml were taken from *D. tertiolecta* cultivations and stock cultures and stored at −20 °C. Prior to DNA extraction samples were pelleted by centrifugation (10,000 × *g*, 10 min) and the supernatant was removed. DNA was extracted from the pellets with PowerSoil™ DNA isolation kit (MO BIO Laboratories, Carlsbad, CA). The extracted DNA sample was used as a template for PCR. Partial eukaryotic 18S rRNA genes of the community were amplified by using primer pair Euk1A and Euk516r-GC [7] and partial bacterial 16S rRNA genes by using primer pair GC-BacV3f [25] and 907r [26]. DGGE was performed as described by Lakaniemi et al. [22]. Sequencing of DGGE bands was conducted at Macrogen Inc. (Seoul, South Korea) and sequence data were analyzed with BioEdit-software and compared with sequences in GenBank. The accession numbers of the 16S rRNA gene sequences submitted to GenBank were JF792588–JF792615.

Quantitative PCR was also conducted from the extracted DNA samples. Primers specific for the nuclear 18S rRNA gene of eukaryotes, EUK345f and EUK499r [41], were used for eukaryotic qPCR and primers specific for 16S rRNA gene of bacteria, 27F [23] and 518R [25], for bacterial qPCR. Reactions were performed as described by Lakaniemi et al. [22]. The logarithm of DNA concentration in the sample is inversely related to cycle threshold (C_T), the number of PCR cycles required to cross a certain fluorescence threshold [19, 41]. Thus, the value of $1/C_T$ was used to estimate the amount of algal and bacterial cells in the samples. Further calibration of the qPCR method was not within the scope of this work.

Results

Growth of *D. tertiolecta* in different photobioreactor configurations

The temperature in the flat-plate photobioreactors varied between 22.4 and 25.5 °C during the cultivations. The DO concentration remained at 94–100 % saturation, between 7.9 and 8.5 mg l^{-1}. Supersaturation was not detected. The pH varied between 7 and 8 and increased during incubation, whilst after day 15 or 16 the pH decreased slightly in the NM and CM (Fig. 1a).

Growth of *D. tertiolecta* was very similar in all flat-plate reactor configurations based on OD, VSS and chlorophyll *a* (Fig. 1b–d). Some differences were seen in eukaryotic $1/C_T$ values between the reactors during the growth, but the end point $1/C_T$ values were similar in all reactor configurations (Fig. 2a). The highest biomass concentrations were 2.9, 2.1, and 2.9 g VSS l^{-1} in the NM, PM, and CM, respectively. The biomass concentration was lower for PM because the cultivation was ended on day 13, whereas the NM and CM cultivations were continued for 20 days. The VSS concentrations in NM and CM on day 13 were 1.8 and 2.0 g l^{-1}, respectively. The highest growth rates were achieved in all reactor configurations between days 0 and 3. Maximum specific growth rates in the NM, PM and CM were 1.3, 1.2 and 1.4 day^{-1}, respectively.

The average Pearson correlation coefficient (r) for OD and VSS from all reactors was 0.99. Chlorophyll *a* correlated well with OD and VSS only up to day 13 ($r = 0.92$ and 0.93, respectively). Eukaryotic $1/C_T$ had overall r of 0.80, 0.81, and 0.72 with logarithms of OD, VSS and chlorophyll *a*, respectively. Eukaryotic qPCR results leveled out by day 16, but OD and VSS continued to increase beyond this point. This trend suggested that the number of algal cells did not increase but the size (or volume) of individual cells still increased.

DOC in the cultures ranged between 0.8 and 1.8 g l^{-1} (Fig. 1e) and did not correlate well with any of the growth indicators. The corresponding r values for DOC vs. OD, VSS, chlorophyll *a* and eukaryotic $1/C_T$ were 0.65, 0.65, 0.44 and 0.67.

Growth of bacteria in the photobioreactors

Both HPC and $1/C_T$ results showed increase in bacterial numbers in the photobioreactor cultures (Figs. 1f, 2b). Based on bacterial $1/C_T$ data, bacterial numbers were initially very similar in all photobioreactors (Fig. 2b). Based on HPC, bacterial numbers varied between 7.2×10^3 and 2.7×10^4 cfu ml^{-1} at the beginning of the cultivations. The logarithm of HPC results and bacterial $1/C_T$ values had r of 0.77. Bacterial $1/C_T$ indicated that the bacterial numbers leveled off after day 13, whereas HPC indicated significant increase in bacterial numbers after day 16. The bacterial $1/C_T$ data had an overall r of 0.97 with the eukaryotic $1/C_T$ results in all reactor configurations.

Microbial community profiles

Eukaryotic community profiles were similar in all reactor configurations (Fig. 3). PCR amplification with the eukaryotic primers yielded a single band on gel electrophoresis. This was resolved to multiple distinct bands on DGGE. Three major DGGE bands and some fainter bands were visible in all of the cultures. The bands labeled in

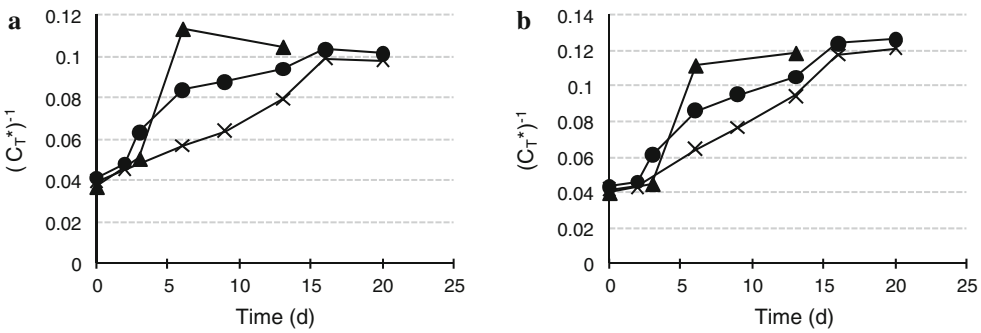

Fig. 2 Results from eukaryotic QPCR (**a**) and bacterial QPCR (**b**) in *circle* the flat plate with no mixer, *triangle* the flat plate with the plain mixer, and *multiplication sign* the flat plate with the complex mixer. *Asterisk* in the $1/C_T$ value indicates that the QPCR values have been normalized to same initial sample volume

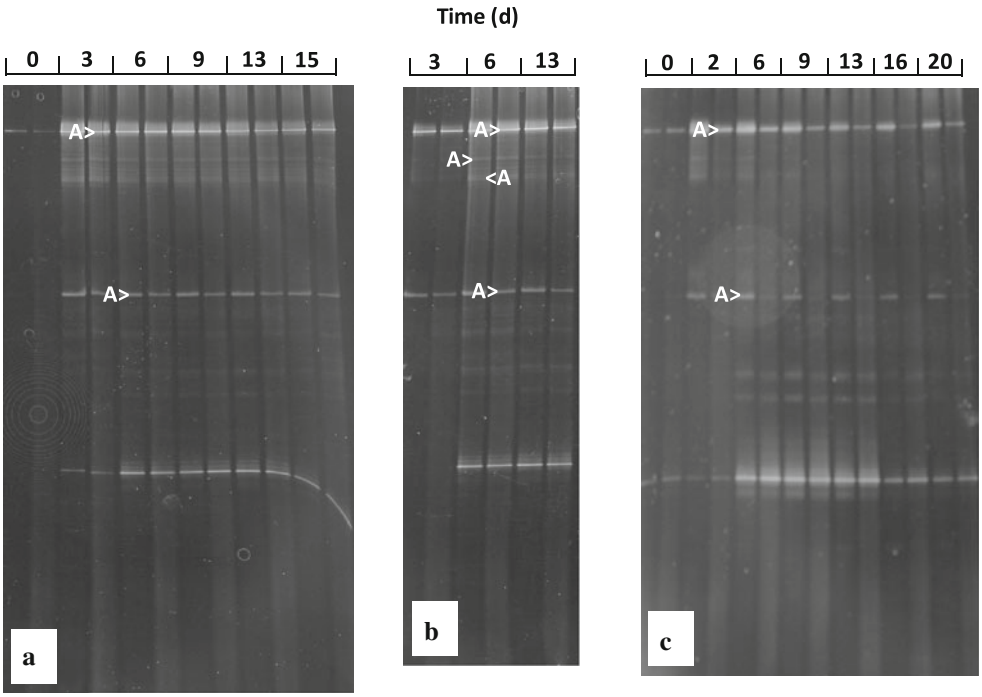

Fig. 3 Eukaryotic community profiles from the flat plate with no mixer (**a**), the flat plate with the plain mixer (**b**), and the flat plate with the complex mixer (**c**) with sampling days shown in the top. All the bands labeled with A had 100 % similarity to *D. tertiolecta* (accession number EF537907)

Fig. 3 were 100 % similar with each other and 100 % similar to *D. tertiolecta* (accession number EF537907). DNA in the lowest bands in Fig. 3 did not yield any PCR product. Thus, *D. tertiolecta* was the dominant eukaryote in the flat-plate cultures.

A total of 26 bacterial sequences were retrieved from the flat-plate reactor cultures. These belonged to the class Gammaproteobacteria (11), Flavobacteria (8), Alphaproteobacteria (6) and an unknown class (1) (Table 1). Some of the bands, such as D, E_9, E_{10}, F_4, F_5, G_5, G_6 and H had a very low similarity to previously described bacteria and may represent novel species (Fig. 4, Table 1).

Bacterial DGGE profiles were relatively stable and reproducible in all of the flat-plate cultivations of *D. tertiolecta* (Fig. 4, Table 1). However, bacteria possibly belonging to the genus *Marinobacter* (band J) and an unknown bacterium (band H) were detected in the CM but not in the other cultures. Some bands, such as B_2, B_3 and B_4 corresponding to *Alcanivorax* spp., disappeared during the incubation. Some bands were visible only in the later phases of algal growth, such as E_6 corresponding *Flavobacterium* spp. and J corresponding *Marinobacter* spp. Most bacteria present in flat-plate cultures of *D. tertiolecta* were also found in the stock culture of *D. tertiolecta* (Fig. 4, Table 1). Bacteria belonging to *Rhodobacteraceae* family were present in all flat-plate cultures but were not detected in the stock culture. However, it is likely that these bacteria were present also in the stock culture but below the detection limit of DGGE.

Discussion

This study monitored growth of non-axenic *D. tertiolecta* in three different flat-plate photobioreactor configurations. All cultures were accompanied with heterotrophic bacteria, likely originating from non-axenic algal inoculum.

The three flat-plate photobioreactor configurations were found to be amenable to *D. tertiolecta* biomass production. Static mixers have been previously reported to enhance microalgal growth [6], but the mixers in the PM and CM did not increase the growth of *D. tertiolecta*. The light intensity used in this study may have been too low to manifest this effect, although the *D. tertiolecta* cells were adapted to low light intensities prior to the experiments. The growth rates were in similar range and biomass concentrations higher than previously reported for *D. tertiolecta* in other studies (Table 2). Efficient growth of *D. tertiolecta* under low light intensity and in the presence of large numbers of associated bacteria is beneficial for industrial utilization of *D. tertiolecta* biomass. Light availability on a global basis is limited in large portions of Earth over most of the year.

The concentration of chlorophyll *a* correlated to some extent with OD and VSS but it decreased after 13 days. The decrease in chlorophyll *a* reflects nutrient limitation [2]. The modified NORO medium has initially 9.9 mM NO_3^- and therefore N limitation is unlikely, but only 0.26 mM phosphate. In comparable growth experiments most of dissolved P was consumed by *D. tertiolecta* by day 7 (data not shown).

Table 1 Selected band identities and affiliations of the flat-plate cultures from DGGE conducted with samples amplified with the bacterial specific PCR primers

Band label (acc)[a]	SL[b]	Sim (%)[c]	Affiliation (acc)[d]	Class/family	Origin of the sample with the closest match
A[e]	364	75.0	Uncultured bacterium (GQ848402)	Unknown/unknown	Sediments from deep-sea hydrothermal vents
B_1 (JF792588)	469	94.7	*Alcanivorax jadensis* (FJ218425)	Gammaproteobacteria/ *Alcanivoracaceae*	Oil-degrading bacteria isolated from the Atlantic Ocean
B_2 (JF792589)	465	97.8			
B_3 (JF792590)	458	100	*Alcanivorax* sp. (EU052767)	Gammaproteobacteria/ *Alcanivoracaceae*	Hydrocarbon-degrading bacteria associated with marine microalgae
B_4 (JF792591)	452	99.8			
C[e]	437	80.1	*Muricauda* sp. (EU839357)	Flavobacteria/*Flavobacteriaceae*	Bacteria associated with filamentous marine cyanobacteria
D (JF792592)	309	85.1	Uncultured bacterium (GQ340102)	Unknown/unknown	Marathonas Reservoir
E_1 (JF792593)	455	98.5	*Flavobacterium* sp. (AF386740)	Flavobacteria/*Flavobacteriaceae*	Non-axenic *Aureococcus anophagefferens* culture
E_2 (JF792594)	471	100			
E_3 (JF792595)	465	100			
E_4 (JF792596)	481	98.5			
E_5 (JF792597)	497	99.6			
E_6^e	505	79.8			
E_7 (JF792598)	495	99.8			
E_8 (JF792599)	465	92.9			
E_9^e	438	83.3			
E_{10} (JF792600)	414	88.4			
E_{11} (JF792601)	474	99.8			
F_1 (JF792602)	481	100	*Halomonas variabilis* (AM945682)	Gammaproteobacteria/ *Halomonadaceae*	Tunisian solar saltern
F_2 (JF792603)	427	92.5	*Halomonas* sp. (EU308361)	Gammaproteobacteria/ *Halomonadaceae*	A solar saltern
F_3 (JF792604)	408	90.7			
F_4 (JF792605)	521	88.5			
F_5^e	472	81.8	*Halomonas* sp. (FJ984872)	Gammaproteobacteria/ *Halomonadaceae*	Not given
F_6 (JF792606)	470	98.1	*Halomonas* sp. (EU308361)	Gammaproteobacteria/ *Halomonadaceae*	A solar saltern
F_7 (JF792607)	476	100			
F_8 (JF792608)	495	99.6			
G_1 (JF792609)	457	97.4	*Ruegeria mobilis* (HQ338148)	Alphaproteobacteria/ *Rhodobacteraceae*	*Ruegeria mobilis* strain F4122 isolated from seawater
G_2 (JF792610)	453	99.8			
G_3 (JF792611)	376	94.4			
G_4 (JF792612)	451	96.5	*Ruegeria* sp. (FJ984836)/ *Roseobacter* sp. (AY332662)	Alphaproteobacteria/ *Rhodobacteraceae*	Not given/Non-axenic *Pfiesteria piscicida* culture
G_5^e	370	83.2	*Ruegeria* sp. (FJ868596)	Alphaproteobacteria/ *Rhodobacteraceae*	Marine recirculating aquaculture system
H (JF792613)	386	88.6	Uncultured bacterium (HM101020)	Unknown/unknown	Marine sponge *Halichondria oshoro*

Table 1 continued

Band label (acc)[a]	SL[b]	Sim (%)[c]	Affiliation (acc)[d]	Class/family	Origin of the sample with the closest match
I (JF792614)	442	90.0	Uncultured bacterium (HM177906)	Unknown/unknown	Crustose coralline algae
J (JF792615)	413	92.0	*Marinobacter* sp. (HQ537165)	Gammaproteobacteria/ *Alteromonadaceae*	Deep-water station ALOHA, North Pacific Ocean

[a] Band label in Fig. 4 with a GenBank accession number

[b] Sequence length

[c] Similarity (%)

[d] Closest species in GenBank database with an accession number

[e] Sequence quality was not high enough to obtain accession number from GenBank

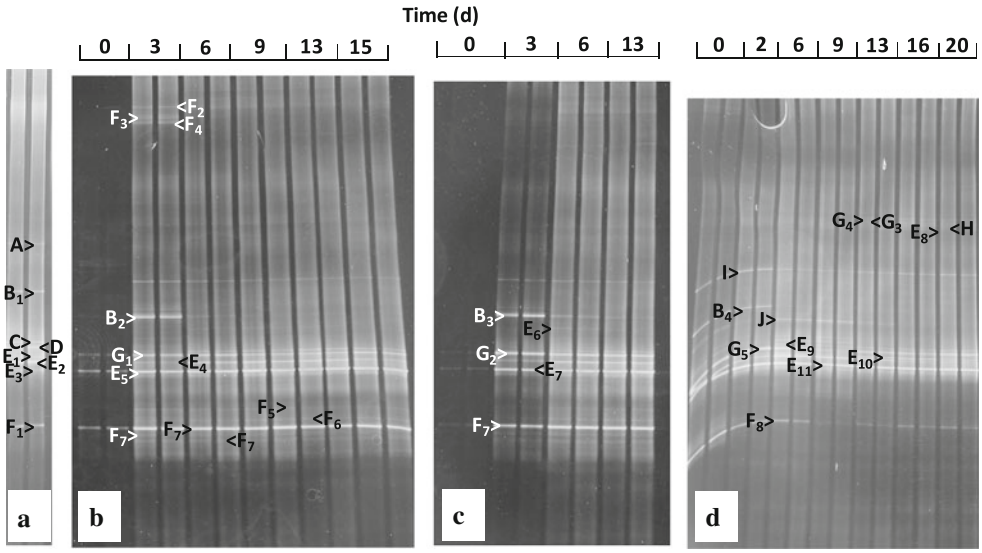

Fig. 4 Bacterial community profiles from *D. tertiolecta* stock culture (**a**), the flat plate with no mixer (**b**), the flat plate with the plain mixer (**c**) and the flat plate with the complex mixer (**d**) with sampling days shown in the top. See Table 1 for the labeled bands

Bacteria utilize organic material exuded by microalgal cells for their growth [39], and in the previous study with *C. vulgaris* positive correlation was observed between DOC and bacterial growth in the NM and PM [22]. The initial DOC of *D. tertiolecta* cultures was relatively high because the NORO medium contained TRIS and EDTA as well as organics introduced with the inoculum. The high background of DOC masked possible associations between microalgal or bacterial growth and DOC.

Eukaryotic qPCR results showed positive correlation with OD and VSS values, both in this and in the previous study with *C. vulgaris* [22]. Also bacterial qPCR results showed moderate positive correlation with HPC in this study. However, bacterial qPCR results indicated that the bacterial numbers leveled off after day 13, whereas HPC increased still after day 16. This may indicate that total number of bacteria did not change much after day 13, whereas there was a shift in the population towards bacteria able to grow on solid agar while the algal growth slowed down. The usefulness of HPC is debatable as the technique gives no insight on the proportion of heterotrophic bacteria capable of growing on the plates. No substantial changes in the bacterial DGGE profiles were seen towards the end of the time course (Fig. 4).

Based on eukaryotic DGGE profiles and microscopic examination, *D. tertiolecta* was the only eukaryote in the flat-plate cultures. *D. tertiolecta* was obtained from the culture collection as a non-axenic culture (containing heterotrophs). The bacteria detected in photobioreactor cultures were generally similar to those found in the stock cultures (Fig. 4). Bacteria typical in tap water were not found in *D. tertiolecta* culture samples [For comparison, see 22]. All bacterial genera identified in the *D. tertiolecta* cultures include halotolerant or halophilic species.

The same major bacterial DNA bands were detected in all DGGE gels from flat-plate culture samples. Bacterial communities in *D. tertiolecta* cultures were dominated by bacteria belonging to classes Gammaproteobacteria, Flavobacteria, and Alphaproteobacteria. These bacterial classes are typical in association with many marine diatoms and dinoflagellates [9, 31, 33, 35]. However, even closely related microalgae have shown to harbor distinct bacterial

Table 2 Maximum specific growth rates and biomass concentrations of *D. tertiolecta*

Maximum specific growth rate (day^{-1})	Maximum biomass concentration ($g\ l^{-1}$)	Bioreactor system	Reference
1.3	2.9	Flat-plate with no mixer	This study
1.2	2.1	Flat-plate with the plain mixer	This study
1.4	2.9	Flat-plate with the complex mixer	This study
1.92	n.a.	Bubble column	[1]
0.67	n.a.	Flat Rouxe's culture flask	[12]
n.a.[a]	1.25	Jar fermentor	[13]
n.a.	0.5	Cylindrical polyethylene PBR[b]	[14]
1.19	n.a.	Flat-plate PBR	[16]
1.85	n.a.	Flat-plate PBR	[17]
n.a.	1.03	Erlenmeyer flask	[38]

[a] *n.a.* data not available

[b] *PBR* photobioreactor

communities [35]. *Roseobacter* and *Halomonas* spp. have repeatedly been detected in cultures of marine microalgae, but other bacterial phylotypes detected in *D. tertiolecta* cultures have not generally been reported to grow in association with other marine microalgae [33, 35]. *Alcanivorax* spp. are alkane-degrading marine bacteria, which have often become prominent in oil-contaminated sea areas if enough nitrogen and phosphorus is available [11, 20]. In this study, *Alcanivorax* spp. were present in all flat-plate cultures up to day 3 after which phosphorus level likely became growth limiting for *Alcanivorax* spp. and resulted in fainter DGGE bands. Due to higher salinity of the medium (3 vs. <0.05 %) the associated bacterial communities in *D. tertiolecta* cultivations were entirely different compared to *C. vulgaris* cultivations in the previous study [22].

Sequence analysis also revealed possible public health concerns in *D. tertiolecta* cultures. Some *Halomonas* spp. such as *Halomona stevensii*, *Halomona johnsoniae* and *Halomona hamiltonii* are potential human pathogens [37]. However, the sequence data for *Halomonas* spp. did not disclose species-level information. *Flavobacterium* spp., on the other hand, have shown antagonistic effects against many human pathogens such as *Staphylococcus aureus*, *Salmonella typhi* and *Vibrio cholerae* [18].

Microalgal growth can sometimes suddenly fail and often the reason for disturbed microalgal biomass production may have been excessive growth of heterotrophic microorganisms, for example, due to competition for nutrients [15]. Elimination of these situations requires understanding of bacterial communities in microalgal growth units. Further, the heterotrophic organisms may provide an untapped resource in microalgal cultures. Fundamental understanding of the community structures and the interactions of the different organisms in microalgal growth units can be used to enhance microalgal biomass production. For example, heterotrophic bacteria could be used to reduce oxygen tension in closed photobioreactors. Ideally, such heterotrophs should not excessively compete with the algae for nutrients and should not produce compounds inhibitory to microalgal growth [5, 24].

Conclusions

Growth rates (1.2–1.4 day^{-1}) and maximum biomass concentrations (2.1–2.9 $g\ l^{-1}$) of *D. tertiolecta* were similar in all three flat-plate photobioreactor configurations. In all photobioreactor configurations, *D. tertiolecta* and bacterial growth (measured with qPCR) correlated with each other. Bacteria associated with *D. tertiolecta* cultures, which were grown in tap water-based media, originated mainly from the non-axenic algal inoculum. High salinity (3 %) of the culture medium inhibited the growth of water borne bacteria from tap water and selected for halotolerant and halophilic bacteria that were initially present in the *D. tertiolecta* inoculum. Heterotrophic communities were relatively stable and reproducible in all flat-plate cultivations and were dominated by bacteria belonging to classes Gammaproteobacteria, Flavobacteria, and Alphaproteobacteria.

Acknowledgments We thank Pertti Vuoriranta for his input to the reactor designs. This study was funded by Finnish Funding Agency for Technology and Innovation (Finland Distinguished Professor Programme, 402/06).

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