



# Lactic acid fermentation of *Arthrospira platensis* (spirulina) biomass for probiotic-based products

Alberto Niccolai<sup>1</sup> · Emer Shannon<sup>2</sup> · Nissreen Abu-Ghannam<sup>2</sup> · Natascia Biondi<sup>1</sup> · Liliana Rodolfi<sup>1,3</sup> · Mario R. Tredici<sup>1</sup>

Received: 19 July 2018 / Revised and accepted: 8 August 2018 / Published online: 8 September 2018  
© The Author(s) 2018

## Abstract

The first objective of this study was to evaluate the use of lyophilised biomass of the cyanobacterium *Arthrospira platensis* F&M-C256 as the sole substrate for lactic acid fermentation by the probiotic bacterium *Lactobacillus plantarum* ATCC 8014. After 48 h of fermentation, the bacterial concentration was 10.6 log CFU mL<sup>-1</sup> and lactic acid concentration reached 3.7 g L<sup>-1</sup>. Lyophilised *A. platensis* F&M-C256 biomass was shown to be a suitable substrate for *L. plantarum* ATCC 8014 growth. The second objective of the study was to investigate whether lactic acid fermentation could enhance in vitro digestibility and antioxidant activity of *A. platensis* biomass. Digestibility increased by 4.4%, however it was not statistically significant, while the antioxidant activity and total phenolic content did increase significantly after fermentation, by 79% and 320% respectively. This study highlights the potential of *A. platensis* F&M-C256 biomass as a substrate for the production of probiotic-based products.

**Keywords** *Arthrospira platensis* · Spirulina · Lactic acid fermentation · Probiotics · Digestibility

## Introduction

Lactic acid-fermented products from milk (yogurt and cheese) are widely consumed, but the demand for dairy-free alternatives is rapidly rising because of the increasing incidence of lactose intolerance and veganism, and as a response to the request for higher nutritional quality and fortified food products (Kandyliis et al. 2016; Panghal et al. 2018). In this respect, lactic acid fermentation is considered a valuable technology to enhance shelf life, safety, sensory and nutritional properties of vegetables and fruits (Di Cagno et al. 2013).

Some algae represent a suitable substrate for the production of fermented foods due to their availability and high nutritional value (Gupta and Abu-Ghannam 2011; Gupta et al. 2011; Uchida and Miyoshi 2013). Many and diverse fermented products (powders, beverages, delicacies) from seaweeds, microalgae (mainly

*Chlorella* and *Dunaliella*) and spirulina (different *Arthrospira* species), usually mixed with plant-derived substrates and obtained through different fermentative processes (by lactic acid bacteria or yeasts or a mixture of these microorganisms), are available in the market and easily found on e-commerce platforms.

In comparison to the numerous studies on algae fermentation (Gupta et al. 2011; Nguyen et al. 2012; Talukder et al. 2012; Uchida and Miyoshi 2013), those on cyanobacteria are limited and focus particularly on *Arthrospira* spp. Bhowmik et al. (2009) added spirulina biomass to cultures of different *Lactobacillus* and *Streptococcus* strains and performed 10h fermentations, observing an increase in the bacterial number with increasing biomass concentrations. Parada et al. (1998) added the filtrate from *A. platensis* cultures to various lactic acid bacteria cultures, which were allowed to ferment for 24 h. The filtrates increased growth of all the bacterial strains. De Caire et al. (2000) added spirulina biomass at different concentrations to milk and then fermented the suspension with a blend of lactic acid bacteria. The presence of spirulina biomass favoured the growth of the bacteria. Several authors (Varga et al. 2002; Guldás and Irkin 2010; Beheshtipour et al. 2012, 2013; Mazinani et al. 2016) have tested the effect of spirulina biomass addition to yogurt, cheese, and fermented milk, with positive results, among which included an increase in the number of lactic acid bacteria and improvement of the nutritional quality of the fermented product during storage. However, to the best of our knowledge, the suitability of spirulina biomass as the sole

✉ Mario R. Tredici  
mario.tredici@unifi.it

<sup>1</sup> Department of Agrifood Production and Environmental Sciences (DISPAA), University of Florence, Piazzale delle Cascine 24, 50144 Florence, Italy

<sup>2</sup> School of Food Science and Environmental Health, Dublin Institute of Technology, Cathal Brugha St, Dublin 1, Ireland

<sup>3</sup> Fotosintetica & Microbiologica S.r.l., Via dei Della Robbia 54, 50132 Florence, Italy

substrate to obtain lactic acid-fermented products has not been investigated.

*Arthrospira*, besides accumulating, like all cyanobacteria, glycogen as primary energy and carbon reserve, contains high levels of proteins (up to 70% dry mass) of high value due to the presence of all the essential amino acids (Becker 2007). *Arthrospira* also contains high levels of vitamins, minerals (particularly iron), essential fatty acids (particularly  $\gamma$ -linolenic acid), carotenoids and chlorophyll (Ghaeni et al. 2014), and a number of unexplored bioactive compounds (Kulshreshtha et al. 2008; Tredici et al. 2009; Chacón-Lee and González-Mariño 2010; Soheili and Khosravi-Darani 2011). *Arthrospira* has potent antioxidant activity due to the presence of polyphenols and phycocyanin (Liu et al. 2011) and also shows interesting lipid-lowering effects (Colla et al. 2008; Bigagli et al. 2017). *Arthrospira* nutritional properties could be improved through lactic acid fermentation, which could increase digestibility of the biomass and availability of nutritious substances, besides providing probiotic bacteria.

Probiotic microorganisms are “live microbial food supplements which beneficially affect the host animal by improving its microbial balance” (Fuller 1992). *Lactobacillus plantarum* has been proposed as probiotic, and several studies have been performed that show its capacity to resist transit through the gastric tract, ability to colonise the host gut, safety and potential health benefits such as cholesterol reduction (de Vries et al. 2006; Georgieva et al. 2009; Karczewski et al. 2010).

A prebiotic is defined as “a substrate that is selectively utilized by host microorganisms conferring a health benefit” (Gibson et al. 2017). Polysaccharides from algae, for example  $\beta$ -glucans from *C. vulgaris*, and spirulina biomass are already recognised and accepted as dietary prebiotics (Jiménez-Escrig et al. 2013; de Jesus Raposo et al. 2016).

This study was designed to investigate the suitability of *A. platensis* F&M-C256 biomass as the sole substrate for *L. plantarum* ATCC 8014 growth and fermentation and to evaluate the fermented product in terms of probiotic bacteria content, in vitro digestibility and antioxidant capacity, which are parameters of great importance for the development of functional foods.

## Materials and methods

### *A. platensis* F&M-C256 biomass production

Biomass of *Arthrospira platensis* F&M-C256 was produced at Archimede Ricerche S.r.l. (Imperia, Italy). The cyanobacterium was cultivated in Zarrouk medium (Zarrouk 1966) in GWP®-I photobioreactors (Chini Zittelli et al. 2013) in semi-batch mode and harvested by filtration. The biomass was washed with tap water to remove excess bicarbonate, then

frozen, lyophilised, powdered and stored at  $-20\text{ }^{\circ}\text{C}$  until use. In the literature, *Arthrospira platensis* is often incorrectly reported as *Spirulina platensis* (Tomaselli et al. 1996). To avoid confusion with the true *Spirulina* genus, in the present work, we have always adopted the correct denomination *A. platensis*.

### Bacterial inoculum preparation

*Lactobacillus plantarum* ATCC 8014 (LAB 8014) was purchased from Cruinn Diagnostics Ltd. (Dublin, Ireland). The culture was maintained at  $-80\text{ }^{\circ}\text{C}$  in a Thermo Scientific Revco Elite PLUS Freezer ULT2586-10-A (USA) in 20% glycerol stocks. For the preparation of the inoculum, 25 mL of autoclaved MRS broth (Scharlau Chemie, Spain) was inoculated with 1 mL of thawed LAB 8014 stock culture and incubated at  $37\text{ }^{\circ}\text{C}$  for 24 h in an orbital shaker (Gallenkamp, Weiss Technik, UK). This inoculum was then diluted (1:100) to obtain a working culture containing  $6\text{--}7\text{ log CFU mL}^{-1}$  as determined by plate counts.

### Fermentation

The suitability of lyophilised *A. platensis* F&M-C256 biomass as substrate for LAB 8014 growth and fermentation was tested according to Gupta et al. (2011). Under sterile conditions, 5 g of lyophilised (8% water content) *A. platensis* F&M-C256 biomass was introduced in a 100 mL Erlenmeyer flask, 40 mL of sterile deionised water was added and the suspension was inoculated with 2 mL of a LAB 8014 culture. Deionised water was then added to reach a final volume of 50 mL. The initial *A. platensis* biomass concentration in the inoculated suspension was thus  $92\text{ g dry weight L}^{-1}$ . Three replicates were prepared. The flasks were incubated at  $37\text{ }^{\circ}\text{C}$  and 100 rpm in an orbital shaker (Innova 42, Mason Technology, Ireland). Samples were taken at 0, 24, 48, and 72 h for microbiological and chemical analyses. A period of 72 h was considered sufficient to observe and evaluate the main fermentation parameters (Gupta et al. 2011). The pH was measured at 0 and 72 h. To evaluate in vitro digestibility, antioxidant capacity and total phenolic content, aliquots (15 mL) were taken at time 0 and after 72 h of incubation and lyophilised before analysis. Phenolics were evaluated also after 24 and 48 h of incubation.

To exclude that the indigenous microbiota of *A. platensis* F&M-C256 biomass may have significantly contributed to the bacterial number increase during fermentation, 5 g of lyophilised *A. platensis* F&M-C256 biomass was introduced in 100 mL Erlenmeyer flasks with 50 mL of deionised water without LAB 8014 inoculation, the flasks were incubated as for the inoculated tests and samples were taken at 0, 24, 48 and 72 h for bacterial growth determination by plate count.

## Analytical methods

### Microbiological analyses

Viable LAB 8014 cell counts in the *A. platensis* broth (log CFU mL<sup>-1</sup>) were performed at the start and after 24, 48 and 72 h by plating on MRS agar (Scharlau Chemie, Spain). The fermentation broth was serially diluted (1:10) in Maximal Recovery Diluent (Sigma–Aldrich, Ireland) before plating. The plates were incubated at 37 °C for 36–48 h. The average weight of a *L. plantarum* cell was determined by growing LAB 8014 cultures (1 mL of thawed stock culture) in 100 mL of MRS broth incubated at 37 °C for 48 h. The cell number was determined by total counts with an Improved Neubauer counting chamber for bacteria, and the biomass dry weight was determined following the method reported by Guccione et al. (2014) using membranes with 0.22 µm porosity (Millipore, USA). The average weight of LAB 8014 cells was then calculated by dividing the biomass dry weight by the cell number.

### Lactic acid determination

Samples were collected from the fermented broths at the start and after 24, 48 and 72 h, diluted 1:10 with water and centrifuged at 16,211×g for 15 min at 4 °C. The cell-free supernatant was used to quantify the lactic acid content. Analyses were carried out with a high-performance liquid chromatography (HPLC) system (Waters Alliance, e2695 Separation module, Waters Corporation, USA) equipped with an auto sampler and dual pump controller. The detection system consisted of a Waters-2996 UV detector (210 nm) and a differential refractometer (Waters-410) connected in series. The column (Rezex ROA–Organic acid 5 H+ (8%) 350.0 mm × 7.8 mm; Phenomenex, UK) was maintained at a temperature of 65 °C and was preceded by a guard column (50.0 mm × 7.8 mm; Phenomenex). An isocratic programme was used with a flow rate of 0.9 mL min<sup>-1</sup> with a mobile phase of ammonium phosphate monobasic (0.05 M) (Sigma–Aldrich) adjusted to pH 2.4 with concentrated phosphoric acid (Sigma–Aldrich). An injection volume of 20 µL was used. Each sample was injected twice. Samples and mobile phases were pre-filtered using 0.22 µm filters (Millipore). Data acquisition and integration were performed using Empower 4.0 software (Waters Corporation). Sodium lactate standards (Sigma–Aldrich) were used to identify and quantify lactic acid in the samples.

### In vitro digestibility

The in vitro digestibility was evaluated by the method of Boisen and Fernández (1997), modified by Niccolai et al. as reported in Batista et al. (2017), on lyophilised fermentation broth at time 0 and after 72 h of fermentation. The method

reproduces the chemical-enzymatic digestion (by gastric and pancreatic juices) that occurs in the proximal tract of the monogastric digestive system. After 72 h of fermentation, broth samples were collected, frozen, lyophilised and powdered (particle size ≤ 1 mm) before analysis. One-gram samples were weighed and transferred to 250mL conical flasks. The analysis comprised two steps of enzymatic digestion: the first performed with porcine pepsin (Applichem, Germany) and the second with porcine pancreatin (Applichem) (Batista et al. 2017).

### DPPH assay

To evaluate the radical scavenging capacity of the fermentation broth at time 0 and 72 h, the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay was carried out according to Rajauria et al. (2013). Briefly, the assay was performed in 96-well microtiter plates (Greiner Bio-One International GmbH, Germany) with 100 µL of DPPH radical solution (165 µM, in methanol, Sigma–Aldrich) and 100 µL of sample (0.2 g of lyophilised broth extracted for 30 min in 5 mL of a 1:5 methanol/water solution). The reaction mixtures were incubated in darkness at 30 °C for 30 min. The absorbances were measured at 517 nm using a UV-Vis spectrophotometric plate reader (BioTek USA).

### Total phenolic content determination

The total phenolic content assay was carried out according to Ganesan et al. (2008) using the Folin Ciocalteu assay. Samples of 0.1 g of lyophilised fermentation broth at time 0 and 72 h were dissolved in 10 mL of deionised water. To 100 µL aliquots of each sample, 2 mL of 2% sodium carbonate (Sigma–Aldrich) in water was added. After 2 min, 100 µL of 50% Folin Ciocalteu reagent (Sigma–Aldrich) was added. The reaction mixture was incubated in darkness at 25 °C for 30 min. The absorbance of each sample was measured at 720 nm using a UV-Vis spectrophotometric microplate reader. Results were expressed in gallic acid equivalents (mg GAE g<sup>-1</sup>) through a calibration curve of gallic acid (0 to 500 µg mL<sup>-1</sup>) (Sigma–Aldrich).

### Statistical analysis

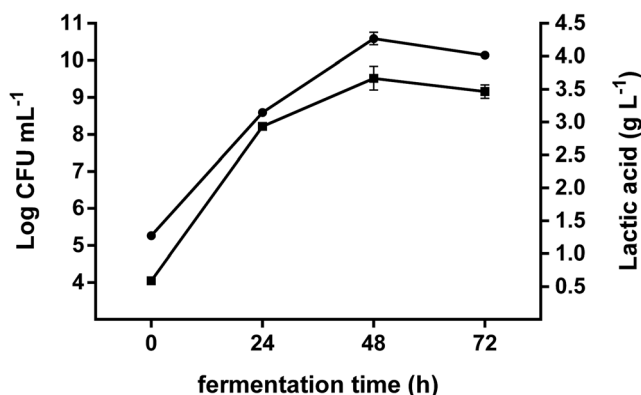
All analyses were conducted in triplicate on three fermentation broths. The results were expressed as mean ± SD (standard deviation). All statistical analyses were carried out using Statgraphics Centurion XV (StatPoint Technologies Inc., USA). Statistical differences between different broths were determined using ANOVA followed by multiple range tests to determine the least significant difference (LSD). Differences were considered statistically significant when  $P < 0.05$ .

## Results and discussion

### Suitability of *A. platensis* F&M-C256 biomass for *L. plantarum* ATCC 8014 growth and lactic acid fermentation

*Lactobacillus plantarum* ATCC 8014 was cultivated for 72 h in a broth containing lyophilised biomass of *A. platensis* F&M-C256 (92 g dry weight L<sup>-1</sup>) as the sole component. The growth curve of LAB 8014, expressed as log CFU per mL of fermentation broth, is shown in Fig. 1. Immediately after inoculation (time zero), an average bacterial concentration of 5.3 ± 0.05 log CFU mL<sup>-1</sup> was measured. The maximal bacterial concentration (10.6 ± 0.2 log CFU mL<sup>-1</sup>) was reached after 48 h of fermentation (Fig. 1). This corresponds to 11.6 ± 0.2 log CFU per gram of *Arthrospira* dry biomass initially added. Considering the average weight of a LAB 8014 cell determined in this study (0.48 ± 0.07 pg), we can assume that after 48 h of fermentation, a concentration of 19.7 ± 7.6 g of bacterial biomass per litre of broth was reached. Thus, the yield of probiotic bacterial biomass from *A. platensis* F&M-C256 biomass was higher than 20% (21 ± 8.3%). These data indicate that *A. platensis* F&M-C256 is a suitable substrate for *L. plantarum* growth.

Several authors (Gardner et al. 2001; Bergqvist et al. 2005; Yoon et al. 2006) have measured the growth of lactic acid bacteria on conventional organic matrices such as beet, onion, carrot and cabbage. From these studies, the maximum concentrations reached by various species of *Lactobacillus* varied in the range of 9–10 log CFU mL<sup>-1</sup>. These concentrations were reached after 24 h in carrot juice (Bergqvist et al. 2005), 48 h in cabbage juice (Yoon et al. 2006) and after 72 h in a vegetable mixture (Gardner et al. 2001). It is worth mentioning that in the above studies, *Lactobacillus* initial concentration was always higher than in the present study. The autochthonous microbial population of raw vegetables and fruits fluctuates



**Fig. 1** Growth curve (circles) of and lactic acid production (squares) by LAB 8014 in a broth containing *A. platensis* F&M-C256 biomass as the sole substrate. The amount of biomass at the start of the experiment (time 0) was 92 g (dry weight) L<sup>-1</sup>. Values are expressed as means ± SD

between 5 and 7 log CFU g<sup>-1</sup>, of which lactic acid bacteria represent a minor part (2–4 log CFU g<sup>-1</sup>) (Di Cagno et al. 2013). In the present study, with the sole lyophilised *A. platensis* F&M-C256 biomass suspended in water and without inoculum, bacterial concentrations of 2.3 and 6.9 log CFU g<sup>-1</sup> were detected at the start and at the end (72 h) of the fermentation, respectively. This indicates that the indigenous microbiota of *A. platensis* F&M-C256 biomass did not significantly affect bacterial number enumeration and the values found by plate count in the inoculated broth were essentially due to LAB 8014. In accordance with the results obtained in the present study, Bhowmik et al. (2009) found that the addition of *A. platensis* (up to 10 g L<sup>-1</sup>) to different *Lactobacillus* cultures promoted growth up to a maximum of 9 log CFU mL<sup>-1</sup> after 10 h, starting from a concentration of 2–3 log CFU mL<sup>-1</sup>. The positive effects of *A. platensis* on the growth of lactic acid bacteria can be attributed to the highly accessible and nutritious substances it contains, which stimulate bacterial metabolism (Beheshtipour et al. 2013). These substances include exopolysaccharides, free amino acids and essential vitamins and minerals (Parada et al. 1998). To observe a positive health effect of probiotic consumption, a minimum level of live microorganisms is required (Vanderhoof and Young 1998). This level, depending upon the strain used and the desired health effect, is usually between 8 and 10 log CFU per day for 1 to 2 weeks (Vanderhoof and Young 1998). Nobaek et al. (2000) found that patients with irritable bowel syndrome (IBS) had an imbalance in their normal colonic flora and exhibited a continuous improvement in overall gastrointestinal function during 12 months following assumption of 400 mL per day of a fermented drink containing 7.7 log CFU mL<sup>-1</sup> of *L. plantarum*. Considering the high concentration of LAB 8014 cells (10.6 log CFU mL<sup>-1</sup>) in the *A. platensis* F&M-C256 fermented broth, we can conclude that this cyanobacterium is promising for the development of fermented foods rich in probiotics.

Lactic acid concentration in the broth during the fermentation process is reported in Fig. 1. Most of lactic acid accumulation (2.93 ± 0.02 g L<sup>-1</sup>) occurred in the first 24 h of fermentation and the maximum concentration was reached after 48 h (3.67 ± 0.18 g L<sup>-1</sup>) with a conversion yield of about 40 mg of lactic acid per gram of dry *A. platensis* biomass. A decrease of pH to values of about 5 was also observed. Considering that *L. plantarum* has a facultative heterofermentative metabolism, the excretion of acetic acid (not analysed in this study), in addition to lactic acid, might have contributed to pH decrease during fermentation.

Common cereal grains have been studied for their ability to support fermentation by lactic acid bacteria. For example, Tanaka et al. (2006) found that the homofermentative *L. delbrueckii* IFO 3202 was able to produce 28 g L<sup>-1</sup> lactic acid from 100 g L<sup>-1</sup> of rice bran when coupled with enzymatic hydrolysis. Although a similar concentration of substrate

(92 g L<sup>-1</sup> of *A. platensis* F&M-C256 biomass) was used in the present study, a maximum lactic acid concentration of less than 4 g L<sup>-1</sup> was obtained. The greater yield of lactic acid with rice bran may be explained by the quick release of sugars following enzyme addition, but also by the higher carbohydrate content and different carbohydrate profile of rice bran (11.3% cellulose + hemicellulose and 46.7% starch + dextrin), probably more suitable to sustain *Lactobacillus* lactic acid fermentation, compared to *A. platensis* F&M-C256 biomass (13.4% total carbohydrates). Uchida and Miyoshi (2013) reported a lactic acid production in the range of 1.5–5.4 g L<sup>-1</sup> from microalgae such as *Chlorella*, *Tetraselmis* and *Nannochloropsis*. Hwang et al. (2012) investigated the lactic acid fermentation of an *Ulva* (*Enteromorpha*) *prolifera* hydrolysate using different *Lactobacillus* strains. Similar to the present study, the lactic acid produced over 24 h of incubation by *L. rhamnosus* was 4.3 g L<sup>-1</sup>, and a similar production (around 4 g L<sup>-1</sup>) was observed also for *L. salivarius*. Fermentation of Irish brown seaweeds (Gupta et al. 2011, 2012) led to a lactic acid production comparable to that found in the present study with *A. platensis* F&M-C256 biomass. It is to note that *A. platensis*, like some micro- and macroalgae, favours more growth of lactic acid bacteria than lactic acid production.

### In vitro digestibility of fermented *A. platensis* F&M-C256 biomass

When the potential application of new matrices for the production of foods is proposed, the digestibility is a parameter which needs to be considered. A two-step (first with pepsin then with pancreatin) digestion protocol was used in this study to assess the in vitro digestibility of the unfermented and fermented (72 h) *A. platensis* F&M-C256 biomass. Fermentation did not improve digestibility significantly ( $P > 0.05$ ), however there was an increase of 4.4% (Table 1).

*Arthrospira platensis* cell wall structure is typical of Gram-negative bacteria with a thin peptidoglycan layer (Tomaselli 1997). The easily breakable cell of *A. platensis* favours the access of the digestive enzymes to the intracellular content, and for this reason, chemical or physical processing steps are in general not required to improve spirulina biomass digestibility (Becker 2007, 2013). Mišurcová et al. (2010) evaluated the in vitro digestibility of *A. platensis* and *Spirulina Pacifica*® produced by Cyanotech

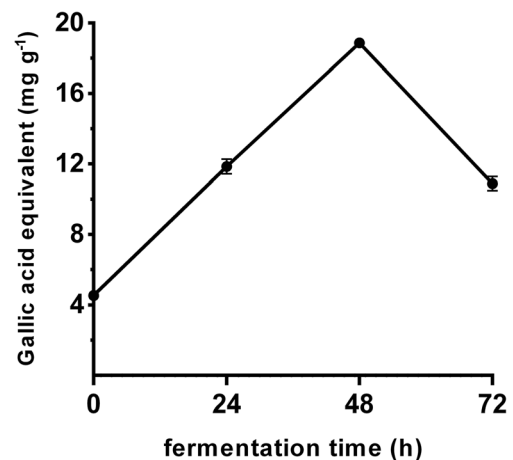
Corp. (USA), finding a higher digestibility value both for *A. platensis* (94.3%) and *Spirulina Pacifica*® (85.6%) compared to that obtained in the present study for *A. platensis* F&M-C256 biomass (about 75%). It is, however, worth pointing out that at the end of the fermentation, a large fraction of the solid residue in the broth was composed of bacterial cells, which have low digestibility (Alfano et al. 2015).

### Antioxidant capacity and phenolic content of fermented *A. platensis* F&M-C256 biomass

*Arthrospira* has in vitro and in vivo antioxidant, radical scavenging and anti-inflammatory activities, principally due to the presence of phycocyanin and polyphenols (Bhat and Madyastha 2000; Abd El-Baki et al. 2009; Liu et al. 2016).

The DPPH radical scavenging capacity of fermented (72 h) and unfermented (0 h) *A. platensis* F&M-C256 broths is shown in Table 1. Fermentation brought to an increase of about 80% in DPPH inhibition. Similar to the results obtained in the present study, Liu et al. (2011) reported an increase of DPPH radical scavenging capacity after fermentation of *A. platensis* biomass in milk.

Phenolic compounds are considered major contributors to antioxidant capacity (Abd El-Baki et al. 2009). Total phenolic content of *Arthrospira* spp. varies from 4.5 to about 50 mg GAE g<sup>-1</sup> depending on strain and culture conditions (Abd El-Baki et al. 2009; Liu et al. 2011; Kepekçi and Saygideger 2012). Liu et al. (2011) found that milk with added *A. platensis* biomass reached 33.6 mg GAE g<sup>-1</sup> after 48 h of fermentation and hypothesised that lactic acid fermentation helps the release of polyphenols from spirulina. In the present study, the total phenolic content of the fermentation broth increased from 4.5 at the start to 18.9 mg GAE g<sup>-1</sup> after 2 days of fermentation and then decreased to 10.9 mg GAE g<sup>-1</sup> after 72 h (Fig. 2). The presence of phenolics could be one of the main reasons for the



**Fig. 2** Total phenolic content, expressed as mg of gallic acid equivalent (GAE) per g of dry fermentation broth initially containing 92 g (dry weight) L<sup>-1</sup> of *A. platensis* F&M-C256 biomass. Values are expressed as means ± SD

**Table 1** In vitro digestibility (% dry matter) and DPPH radical scavenging capacity (%) of lyophilised fermentation broth at the start and at the end of fermentation. Values are expressed as mean ± SD. Different letters denote a significant difference ( $P < 0.05$ )

Fermentation times (h)	In vitro digestibility (%)	DPPH radical scavenging capacity (%)
0	74.3 ± 0.9 <sup>a</sup>	20.5 ± 1.7 <sup>a</sup>
72	77.6 ± 3.6 <sup>a</sup>	36.6 ± 1.2 <sup>b</sup>

antioxidant activity of the fermented *A. platensis* broth, possibly together with phycocyanin (not addressed in this study). Further studies are necessary to fully clear this point.

Many studies report that *L. plantarum* strains present antioxidant activity (Li et al. 2012; Kachouri et al. 2015). Suzuki et al. (2013) identified two compounds, L-3-(4-hydroxyphenyl) lactic acid and L-indole-3-lactic acid, as possible cause of DPPH radical scavenging capacity of *L. plantarum* cultures. It is worth noting that in this work, the broth after 72-h fermentation contained a high concentration of LAB 8014 that could be responsible, together with phenolics and phycocyanin released from *A. platensis* F&M-C256, for the increase in radical scavenging capacity.

## Conclusions

In conclusion, this study shows that *A. platensis* F&M-C256 is a suitable substrate for *L. plantarum* ATCC 8014 growth. At the end of the fermentation, *L. plantarum* ATCC 8014 cells constitute more than 20% of the broth dry weight. While no significant enhancement in digestibility of *A. platensis* biomass was observed following lactic acid fermentation, antioxidant activity was strongly increased (by about 80%). Fermentation by lactic acid bacteria is an appropriate technology to obtain innovative functional products from *A. platensis*, which can provide to the consumer, besides the highly nutritional properties of spirulina further increased by fermentation, a significant amount of probiotic *Lactobacillus* cells, thus conferring additional favourable properties to the final product.

**Acknowledgments** A stay at the Dublin Institute of Technology was funded by the University of Florence Ph.D programme. The authors are indebted to Archimede Ricerche S.r.l (Imperia, Italy) for kindly providing the *A. platensis* biomass.

## Compliance with ethical standards

**Conflict of interest** *A. platensis* F&M-C256 belongs to the Microalgae Culture Collection of Fotosintetica & Microbiologica S.r.l., in which company M.R. Tredici and L. Rodolfi have a financial interest. All the other authors have no conflicts of interest.

**Open Access** This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

## References

Abd El-Baki HH, El Baz FK, El-Baroty GS (2009) Production of phenolic compounds from *Spirulina maxima* microalgae and its protective effects. *Afr J Biotechnol* 8:7059–7067

- Alfano A, Donnarumma G, Cimini D, Fusco A, Marzaioli I, De Rosa M, Schiraldi C (2015) *Lactobacillus plantarum*: microfiltration experiments for the production of probiotic biomass to be used in food and nutraceutical preparations. *Biotechnol Prog* 31:325–333
- Batista AP, Niccolai A, Fradinho P, Fragoso S, Bursic I, Rodolfi L, Biondi N, Tredici MR, Sousa I, Raymundo A (2017) Microalgae biomass as an alternative ingredient in cookies: sensory, physical and chemical properties, antioxidant activity and in vitro digestibility. *Algal Res* 26:161–171
- Becker EW (2007) Microalgae as a source of protein. *Biotechnol Adv* 25: 207–210
- Becker EW (2013) Microalgae for human and animal nutrition. In: Richmond A, Hu Q (eds) *Handbook of microalgal culture*, 2nd edn. Wiley, Oxford, pp 461–503
- Beheshtipour H, Mortazavian AM, Haratian P, Darani KK (2012) Effects of *Chlorella vulgaris* and *Arthrospira platensis* addition on viability of probiotic bacteria in yogurt and its biochemical properties. *Eur Food Res Technol* 235:719–728
- Beheshtipour H, Mortazavian AM, Mohammadi R, Sohrabvandi S, Khosravi-Darani K (2013) Supplementation of *Spirulina platensis* and *Chlorella vulgaris* algae into probiotic fermented milks. *Compr Rev Food Sci F* 12:144–154
- Bergqvist SW, Sandberg AS, Carlsson NG, Andlid T (2005) Improved iron solubility in carrot juice fermented by homo- and hetero-fermentative lactic acid bacteria. *Food Microbiol* 22:53–61
- Bhat VB, Madyastha KM (2000) C-Phycocyanin: a potent peroxy radical scavenger *in vivo* and *in vitro*. *Biochem Biophys Res Commun* 275:20–25
- Bhowmik D, Dubey J, Mehra S (2009) Probiotic efficiency of *Spirulina platensis*-stimulating growth of lactic acid bacteria. *World J Dairy Food Sci* 4:160–163
- Bigagli E, Cinci L, Niccolai A, Tredici MR, Biondi N, Rodolfi L, Lodovici M, D'Ambrosio M, Mori G, Luceri C (2017) Safety evaluations and lipid-lowering activity of an *Arthrospira platensis* enriched diet: a 1-month study in rats. *Food Res Int* 102:380–386
- Boisen S, Fernández JA (1997) Prediction of the total tract digestibility of energy in feedstuffs and pig diets by *in vitro* analyses. *Anim Feed Sci Tech* 68:277–286
- Chacón-Lee TL, González-Mariño GE (2010) Microalgae for “healthy” foods—possibilities and challenges. *Compr Rev Food Sci Food Saf* 9:655–675
- Chini Zittelli G, Rodolfi L, Bassi N, Biondi N, Tredici MR (2013) Photobioreactors for microalgal biofuel production. In: Borowitzka MA, Moheimani NR (eds) *Algae for biofuels and energy*. Springer, Dordrecht, pp 115–131
- Colla LM, Muccillo-Baisch AL, Costa JAV (2008) *Spirulina platensis* effects on the levels of total cholesterol, HDL and triglycerides in rabbits fed with a hypercholesterolemic diet. *Braz Arch Biol Technol* 51:405–411
- de Caire GZ, Parada JL, Zaccaro MC, Stomi de Cano MM (2000) Effect of *Spirulina platensis* biomass on the growth of lactic acid bacteria in milk. *World J Microbiol Biotechnol* 16:563–565
- de Jesus Raposo MF, de Morais AMMB, de Morais RMSC (2016) Emergent sources of prebiotics: seaweeds and microalgae. *Mar Drugs* 14:1–27
- de Vries MC, Vaughan EE, Kleerebezem M, de Vos WM (2006) *Lactobacillus plantarum*—survival, functional and potential probiotic properties in the human intestinal tract. *Int Dairy J* 16:1018–1028
- Di Cagno R, Coda R, De Angelis M, Gobbetti M (2013) Exploitation of vegetables and fruits through lactic acid fermentation. *Food Microbiol* 33:1–10
- Fuller R (1992) History and development of probiotics. In: Fuller R (ed) *Probiotics*. Springer, Dordrecht, pp 1–8
- Ganesan P, Kumar CS, Bhaskar N (2008) Antioxidant properties of methanol extract and its solvent fractions obtained from selected Indian red seaweeds. *Bioresour Technol* 99:2717–2723

- Gardner NJ, Savard T, Obermeier P, Caldwell G, Champagne CP (2001) Selection and characterization of mixed starter cultures for lactic acid fermentation of carrot, cabbage, beet and onion vegetable mixtures. *Int J Food Microbiol* 64:261–275
- Georgieva R, Iliev I, Haertlé T, Chobert J-M, Ivanova I, Danova S (2009) Technological properties of candidate probiotic *Lactobacillus plantarum* strains. *Int Dairy J* 19:696–702
- Ghaeni M, Roomiani L, Moradi Y (2014) Evaluation of carotenoids and chlorophyll as natural resources for food in spirulina microalgae. *Appl Food Biotechnol* 2:39–44
- Gibson GR, Hutkins R, Sanders ME, Prescott SL, Reimer RA, Salminen SJ, Scott K, Stanton C, Swanson KS, Cani PD, Verbeke K, Reid G (2017) Expert consensus document: the International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. *Nat Rev Gastro Hepat* 14:491–502
- Guccione A, Biondi N, Sampietro G, Rodolfi L, Bassi N, Tredici MR (2014) *Chlorella* for protein and biofuels: from strain selection to outdoor cultivation in a Green Wall panel photobioreactor. *Biotechnol Biofuels* 7:84
- Guldas M, Irkin R (2010) Influence of *Spirulina platensis* powder on the microflora of yoghurt and acidophilus milk. *Mljekarstvo* 60:237–243
- Gupta S, Abu-Ghannam N (2011) Bioactive potential and possible health effects of edible brown seaweeds. *Trends Food Sci Technol* 22:315–326
- Gupta S, Abu-Ghannam N, Scannell AG (2011) Growth and kinetics of *Lactobacillus plantarum* in the fermentation of edible Irish brown seaweeds. *Food Bioprod Process* 89:346–355
- Gupta S, Abu-Ghannam N, Rajauria G (2012) Effect of heating and probiotic fermentation on the phytochemical content and antioxidant potential of edible Irish brown seaweeds. *Bot Mar* 55:527–537
- Hwang HJ, Kim SM, Chang JH, Lee SB (2012) Lactic acid production from seaweed hydrolysate of *Enteromorpha prolifera* (Chlorophyta). *J Appl Phycol* 24:935–940
- Jiménez-Escrig A, Gómez-Ordóñez E, Tenorio MD, Rupérez P (2013) Antioxidant and prebiotic effects of dietary fiber co-travelers from sugar kombu in healthy rats. *J Appl Phycol* 25:503–512
- Kachouri F, Ksontini H, Kraiem M, Setti K, Mechmeche M, Hamdi M (2015) Involvement of antioxidant activity of *Lactobacillus plantarum* on functional properties of olive phenolic compounds. *J Food Sci Tech* 52:7924–7933
- Kandyli P, Pissaridi K, Bekatorou A, Kanellaki M, Koutinas AA (2016) Dairy and non-dairy probiotic beverages. *Curr Opin Food Sci* 7:58–63
- Karczewski J, Troost FJ, Konings I, Dekker J, Kleerebezem M, Brummer R-JM, Wells JM (2010) Regulation of human epithelial tight junction proteins by *Lactobacillus plantarum* *in vivo* and protective effects on the epithelial barrier. *Am J Physiol Gastrointest Liver Physiol* 298:G851–G859
- Kepekçi RA, Saygideger SD (2012) Enhancement of phenolic compound production in *Spirulina platensis* by two-step batch mode cultivation. *J Appl Phycol* 24:897–905
- Kulshreshtha A, Jarouliya U, Bhadauriya P, Prasad GBKS, Bisen PS (2008) *Spirulina* in health care management. *Curr Pharm Biotechnol* 9:400–405
- Li S, Zhao Y, Zhang L, Zhang X, Huang L, Li D, Niu C, Yang Z, Wang Q (2012) Antioxidant activity of *Lactobacillus plantarum* strains isolated from traditional Chinese fermented foods. *Food Chem* 135:1914–1919
- Liu JG, Hou CW, Lee SY, Chuang Y, Lin CC (2011) Antioxidant effects and UVB protective activity of *Spirulina* (*Arthrospira platensis*) products fermented with lactic acid bacteria. *Process Biochem* 46:1405–1410
- Liu Q, Huang Y, Zhang R, Cai T, Cai Y (2016) Medical application of *Spirulina platensis* derived C-phycoerythrin. *Evid Based Complement Alternat Med*, Article ID 7803846
- Mazinani S, Fadaei V, Khosravi-Darani K (2016) Impact of *Spirulina platensis* on physicochemical properties and viability of *Lactobacillus acidophilus* of probiotic UF feta cheese. *J Food Process Pres* 40:1318–1324
- Mišurcová L, Kráčmar S, Bořivoj K, Vacek J (2010) Nitrogen content, dietary fiber, and digestibility in algal food products. *Czech J Food Sci* 28:27–35
- Nguyen CM, Kim J-S, Hwang HJ, Park MS, Choi GJ, Choi YH, Jang KS, Kim J-C (2012) Production of L-lactic acid from a green microalga, *Hydrodictyon reticulatum*, by *Lactobacillus paracasei* LA104 isolated from the traditional Korean food, makgeolli. *Bioresour Technol* 110:552–559
- Nobaek S, Johansson ML, Molin G, Ahrné S, Jeppsson B (2000) Alteration of intestinal microflora is associated with reduction in abdominal bloating and pain in patients with irritable bowel syndrome. *Am J Gastroenterol* 95:1231–1238
- Panghal A, Janghu S, Virkar K, Gat Y, Kumar V, Chhikara N (2018) Potential non-dairy probiotic products—a healthy approach. *Food Biosci* 21:80–89
- Parada JL, de Caire GZ, de Mulé MCZ, de Cano MMS (1998) Lactic acid bacteria growth promoters from *Spirulina platensis*. *Int J Food Microbiol* 45:225–228
- Soheili M, Khosravi-Darani K (2011) The potential health benefits of algae and micro algae in medicine: a review on *Spirulina platensis*. *Curr Nutr Food Sci* 7:279–285
- Rajauria G, Jaiswal AK, Abu-Ghannam N, Gupta S (2013) Antimicrobial, antioxidant and free radical-scavenging capacity of brown seaweed *Himanthalia elongata* from western coast of Ireland. *J Food Biochem* 37:322–335
- Suzuki Y, Kosaka M, Shindo K, Kawasumi T, Kimoto-Nira H, Suzuki C (2013) Identification of antioxidants produced by *Lactobacillus plantarum*. *Biosci Biotech Biochem* 77:1299–1302
- Talukder MMR, Das P, Wu JC (2012) Microalgae (*Nannochloropsis salina*) biomass to lactic acid and lipid. *Biochem Eng J* 68:109–113
- Tanaka T, Hoshina M, Tanabe S, Sakai K, Ohtsubo S, Taniguchi (2006) Production of D-lactic acid from defatted rice bran by simultaneous saccharification and fermentation. *Bioresour Technol* 97:211–217
- Tomaselli L (1997) Morphology, ultrastructure and taxonomy of *Arthrospira* (*Spirulina*) *maxima* and *Arthrospira* (*Spirulina*) *platensis*. In: Vonshak A (ed) *Spirulina platensis* (*Arthrospira*). Phycology, cell biology and biotechnology. Taylor & Francis, London, pp 1–19
- Tomaselli L, Palandri MR, Tredici MR (1996) On the correct use of the *Spirulina* designation. *Algol Stud* 83:539–548
- Tredici MR, Biondi N, Ponis E, Rodolfi L, Chini Zittelli G (2009) Advances in microalgal culture for aquaculture feed and other uses. In: Burnell G, Allan G (eds) *New Technologies in Aquaculture*. Woodhead Publishing, Oxford, pp 610–676
- Uchida M, Miyoshi T (2013) Algal fermentation - the seed for a new fermentation industry of foods and related products. *Jarq-Jpn Agr Res Q* 47:53–63
- Vanderhoof JA, Young RJ (1998) Use of probiotics in childhood gastrointestinal disorders. *J Pediatr Gastr Nutr* 27:323–332
- Varga L, Szigeti J, Kovács R, Földes T, Buti S (2002) Influence of a *Spirulina platensis* biomass on the microflora of fermented ABT milks during storage (R1). *J Dairy Sci* 85:1031–1038
- Yoon KY, Woodams EE, Hang YD (2006) Production of probiotic cabbage juice by lactic acid bacteria. *Bioresour Technol* 97:1427–1430
- Zarrouk C (1966) Contribution à l'étude d'une cyanophycée. Influence de divers facteurs physiques et chimiques sur la croissance et la photosynthèse de *Spirulina maxima* (Setch et Gardner) Geitler. Ph.D. thesis, Université de Paris, France