



UNIVERSITÀ
DEGLI STUDI
FIRENZE

DAGRI
DIPARTIMENTO DI SCIENZE
E TECNOLOGIE AGRARIE,
ALIMENTARI, AMBIENTALI E FORESTALI



“Yogurt di spirulina: le proprietà nutrizionali di spirulina ed i benefici dei batteri lattici”

**Alberto Nicolai¹, Emer Shannon², Nissreen Abu-Ghannam², Natascia Biondi¹,
Liliana Rodolfi^{1,3}, Mario R. Tredici¹**

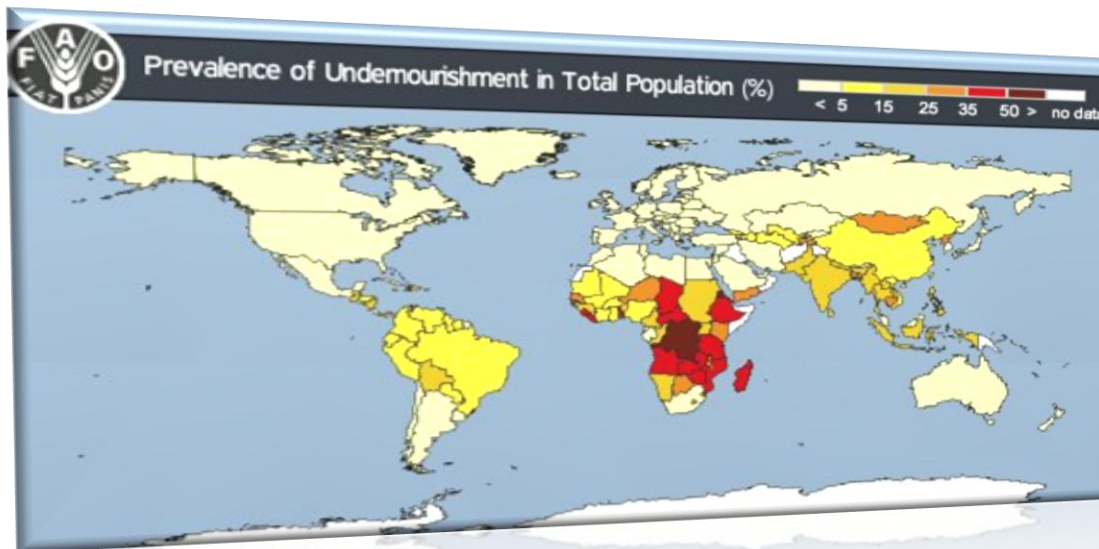
¹ Department of Agrifood Production and Environmental Sciences (DISPAA), University of Florence, Italy;

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³ FoodMicroTeam S.r.l, Florence, Italy

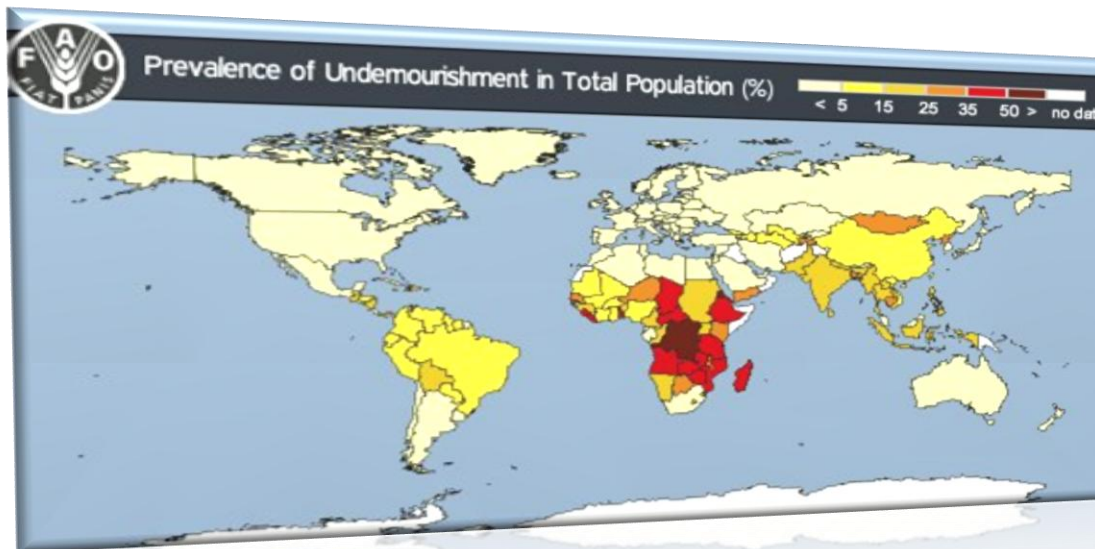
20th November 2019

795 milioni di persone sono cronicamente sottoalimentate



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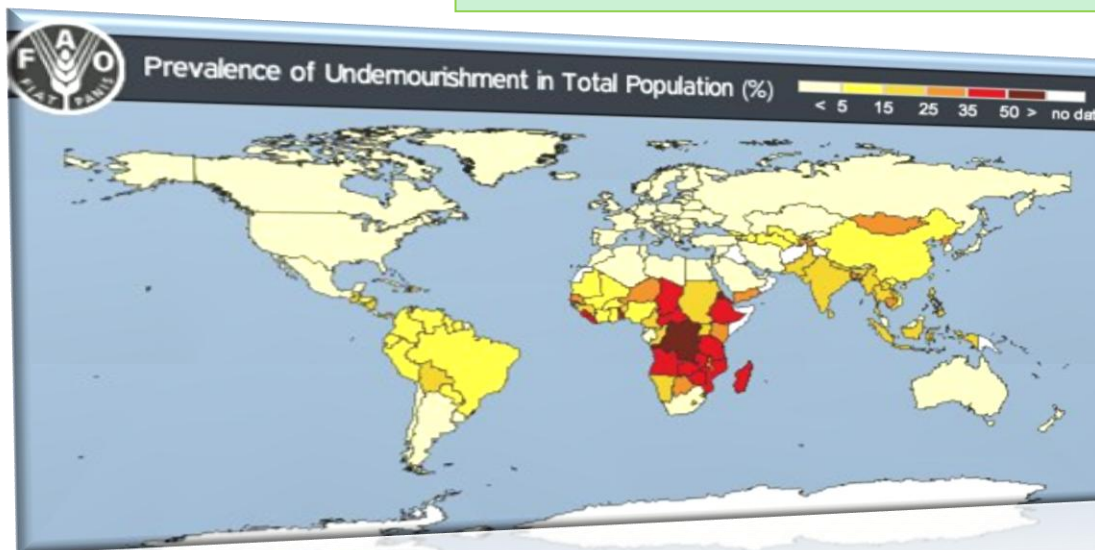
9 miliardi di persone che abiteranno la terra nel 2044 consumeranno materie prime come farebbero oggi 13 miliardi di persone



795 milioni di persone sono cronicamente sottoalimentate

9 miliardi di persone che abiteranno la terra nel 2044 consumeranno materie prime come farebbero oggi 13 miliardi di persone

la produzione mondiale di cibo e materie prime dovrebbe aumentare del **110%** nei prossimi 40 anni



I PRINCIPALI OSTACOLI da superare:

Richieste di acqua dolce in crescita da aree urbanizzate e agricoltura



Cambiamento climatico imputabile alla pressione antropica sull'ambiente

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SFIDA

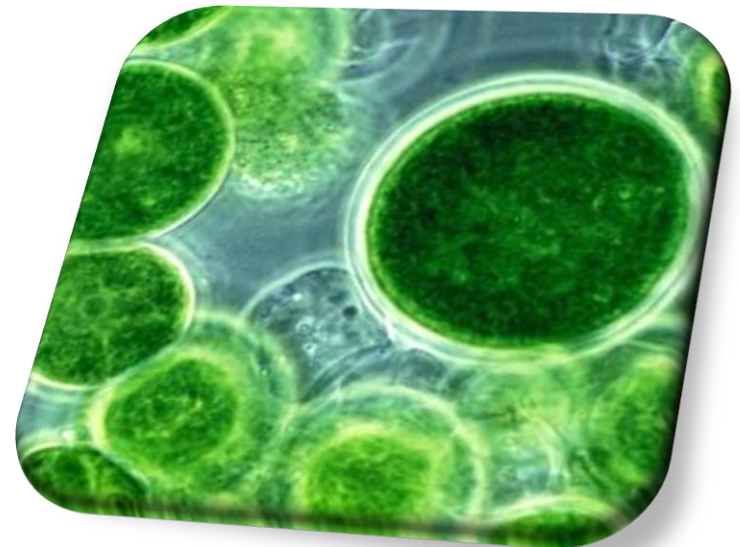


Organismi fototrofi

50% dell'attività fotosintetica sulla terra

COLTURE ALGALI

- buona produttività
- non richiedono terreno fertile
- possono crescere su acqua salata
- non richiedono erbicidi o pesticidi
- sintetizzano co-prodotti di alto valore



APERTI



CHIUSI



Alveolari



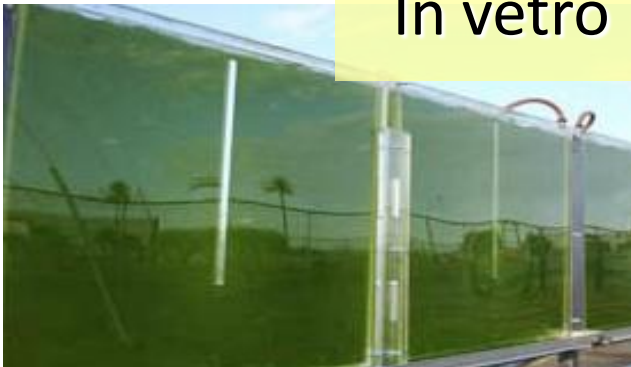
WO 2004/074423 A2

GWPI



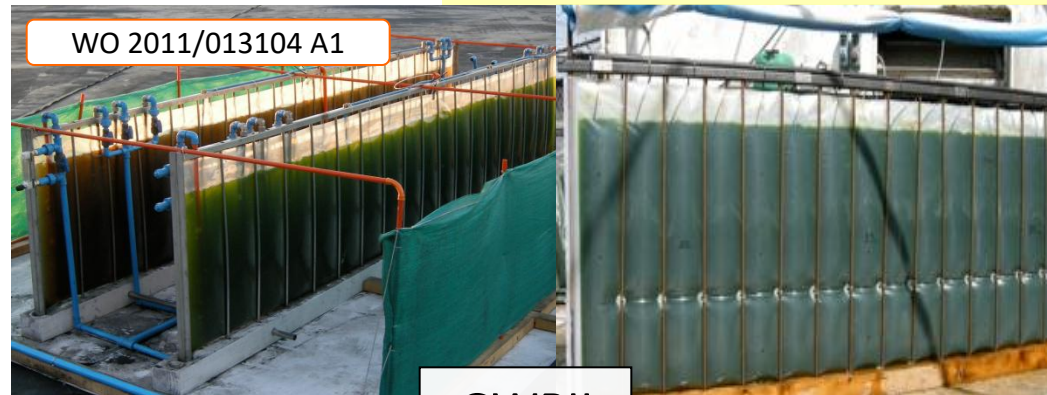
Camera di coltura
usa e getta

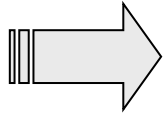
In vetro



WO 2011/013104 A1

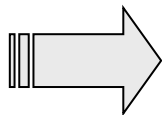
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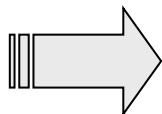


Integratori alimentari (capsule e compresse)





Integratori alimentari (capsule e compresse)



Alimentazione in acquacoltura per molluschi, gamberi, zooplancton



Microalga	Anno di approvazione come cibo nell'EU	Applicazioni e prodotti
<i>Arthrospira platensis</i> (spirulina)	Utilizzato prima del maggio 1997	Nutrizione umana e animale, cosmesi
<i>Chlorella luteoviridis</i>	Utilizzato prima del maggio 1997	Nutrizione umana, acquacoltura, cosmesi
<i>Chlorella pyrenoidosa</i>	Utilizzato prima del maggio 1997	Nutrizione umana, acquacoltura, cosmesi
<i>Chlorella vulgaris</i>	Utilizzato prima del maggio 1997	Nutrizione umana, acquacoltura, cosmesi
<i>Aphanizomenon flos-aquae</i>	Utilizzato prima del maggio 1997	Nutrizione umana
<i>Odontella aurita</i>	Approvato nel 2005	Nutrizione umana, cosmesi
<i>Tetraselmis chuii</i>	Approvato nel 2014	Nutrizione umana
<i>Haematococcus pluvialis</i>	Approvato nel 2014	Acquacoltura, astaxantina

Il mercato alimentare è dominato da pochi generi microalgali

Arthrospira

Chlorella

Dunaliella

Haematococcus

Biomasse o estratti incorporati in pasta, biscotti, pane, snack, caramelle, yogurt, gelati, bevande

Il mercato alimentare è dominato da pochi generi microalgali

Arthrospira
Chlorella
Dunaliella
Haematococcus

Biomasse o estratti incorporati in pasta, biscotti, pane, snack, caramelle, yogurt, gelati, bevande

~ 15.000 t/anno

Prodotto	Specie	Stato
Cibi funzionali e integratori alimentari	<i>Arthrospira</i> (6000-7000 t) <i>Chlorella</i> (4000-7000 t) <i>Dunaliella</i> (1000-1600 t) <i>Haematococcus</i> (280-350 t)	Commerciale (Raceway ponds, circular ponds, lagoons, PBR)

Microalga	Costo biomassa (€/kg)	Mercato (€ Milioni)
<i>Arthrospira</i>	5-50	120-160
<i>Chlorella</i>	12-40	100-130
<i>Haematococcus</i>	60-100	70-110
<i>Dunaliella</i>	150-340	80-100

Quali sono i **componenti cellulari più interessanti e promettenti** per un **futuro sviluppo commerciale di prodotti nutraceutici**, ricchi in **probiotici a base di microalghe e cianobatteri**?

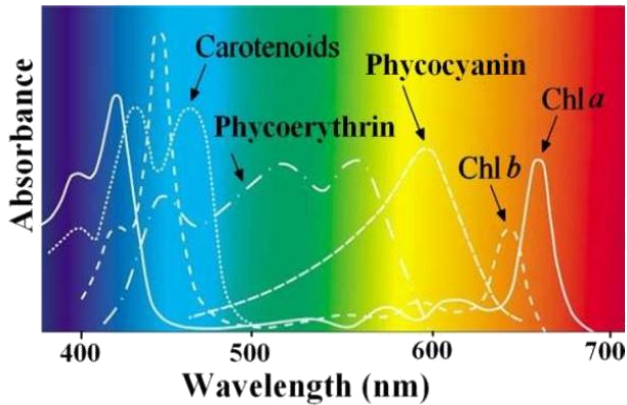
Quali sono i **componenti cellulari** più interessanti e **promettenti** per un **futuro sviluppo commerciale** di **prodotti nutraceutici**, ricchi in **probiotici** a base di **microalghe** e **cianobatteri**?

A large, solid blue downward-pointing arrow connects the question box to the list of components.

Pigmenti

Acidi grassi

Proteine



✓ Pigmenti
Acidi grassi
Proteine

Colore	Specie microalgali	Tipo di pigmento	Contento pigmento (%)
Rosso	<i>Haematococcus pluvialis</i> <i>Chlorella zofingiensis</i>	Astaxantina	Fino al 4
Arancio	<i>Dunaliella salina</i>	β -carotene	Fino al 12
Giallo	<i>Chlorella zofingensis</i> <i>Scenedesmus almeriensis</i> <i>Muriellopsis sp.</i>	Luteina	0.34 0.45 0.43
Marrone	Diatomee e Haptophyta	Fucoxantina	0.6-1.2
Verde	Maggior parte delle Chlorophyta	Clorofilla	1-3
Blu	<i>Arthrospira platensis</i> (spirulina)	C-Ficocianina	10-12



Coloranti nell'industria alimentare



Integratori alimentari con funzione antiossidante



Rendere vivo il colore della carne di salmone



Bevande/cibi funzionali a base di ficocianina

Pigmenti
✓ Acidi grassi
Proteine

**Acidi grassi
essenziali**



PUFAs



Omega-3



Omega-6

EPA e DHA esercitano un'importante effetto sulla salute

Pigmenti
✓ Acidi grassi
Proteine

Acidi grassi
essenziali



PUFAs

Omega-3

Omega-6

EPA e DHA esercitano un importante effetto sulla salute

Molti studi suggeriscono che EPA e DHA

- Ω Supportano salute cardiovascolare (EPA e DHA)
- Ω Esercitano effetti antagonisti contro diverse forme di cancro ed anche contro disturbi infiammatori e autoimmuni (EPA)
- Ω Giocano un ruolo essenziale nello sviluppo di retina e cervello (DHA)



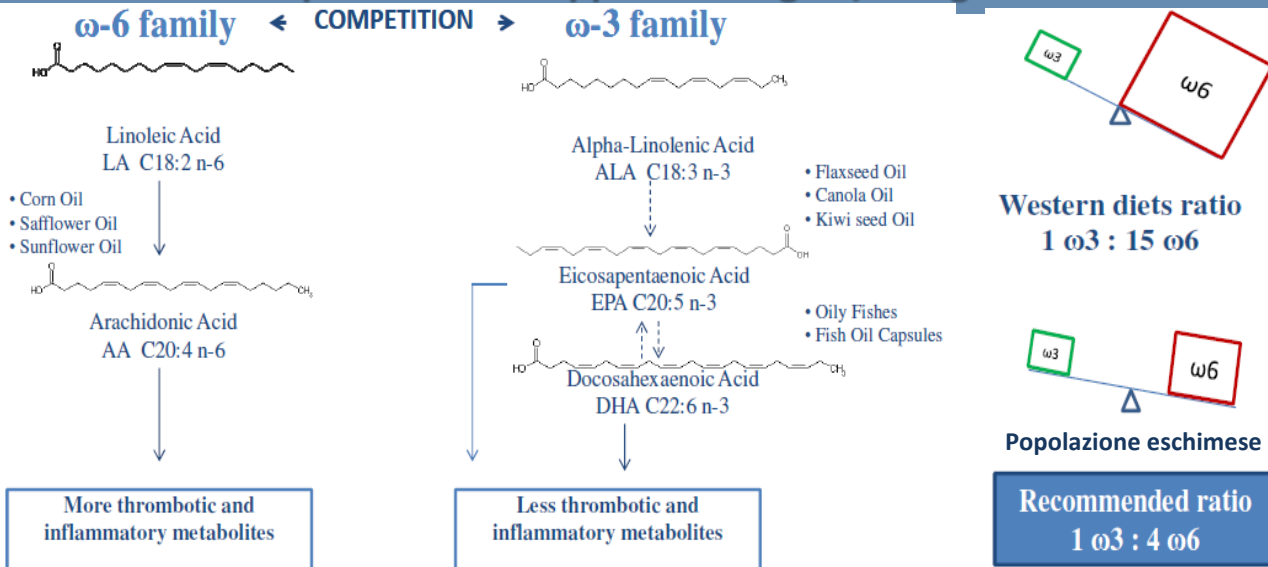
DOSI RACCOMANDATE		
Nuove linee guida EFSA (EU, 2010)		
PUFA	Popolazione	Quantitativo
DHA+EPA	Adulto generico	250* mg/giorno
DHA	Neonati 6 mesi - Bambini 2 anni età	100 mg/giorno
DHA	Donne durante gravidanza e allattamento	100 mg/giorno

* Più del doppio in
pazienti con malattie
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* Più del doppio in pazienti con malattie coronariche

Importanza del rapporto omega-3/omega-6



Pigmenti

Acidi grassi

✓ Proteine

Arthrospira platensis* e *Chlorella vulgaris



Elevato contenuto proteico → sino al 70% (peso secco)

Pigmenti

Acidi grassi

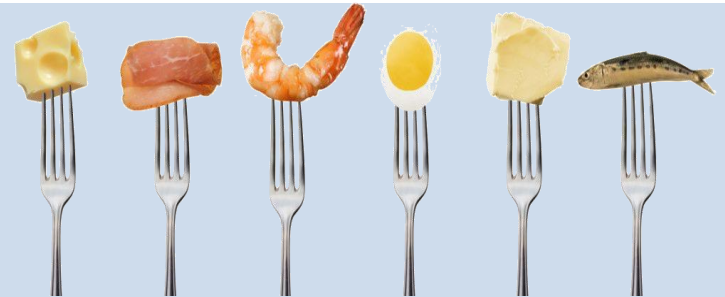
✓ Proteine

Arthrospira platensis e *Chlorella vulgaris*



Elevato contenuto proteico → sino al 70% (peso secco)

→ Possono rappresentare la **parte proteica della dieta**



→ Buoni **sostituti** per le **proteine di origine animale**



Dalla quantificazione
delle proteine
(analizzatore elementare
CHNO)

Assumendo al giorno **5 grammi** di **C256** o **Chlorella vulgaris Allma**  **Arthrospira platensis F&M-** 

la quantità di solito consigliata dalle informazioni nutrizionali per
diversi prodotti a base di *Arthrospira* e *Chlorella*
(Website Aurospirul; Website MyProtein; Website Bulk Powders; Website Health Ranger Store)

Pigmenti
Acidi grassi
✓ **Proteine**

una persona può arricchire la dieta quotidiana fino a **3,5** o **2,8%**
rispettivamente di **proteine non animali**, con ridotto quantitativo di
lipidi (rispettivamente solo 0,5 e 0,8%)

Dalla quantificazione
delle proteine
(analizzatore elementare
CHNO)

Assumendo al giorno **5 grammi** di
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Arthrospira platensis F&M-



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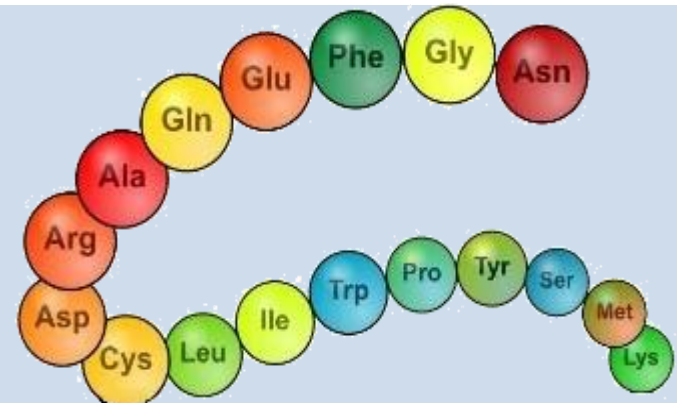
Pigmenti

Acidi grassi

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lipidi (rispettivamente solo 0,5 e 0,8%)

Le biomasse di *Arthrospira* e *Chlorella* contengono
la maggior parte degli **aminoacidi essenziali**



Pigmenti

Acidi grassi

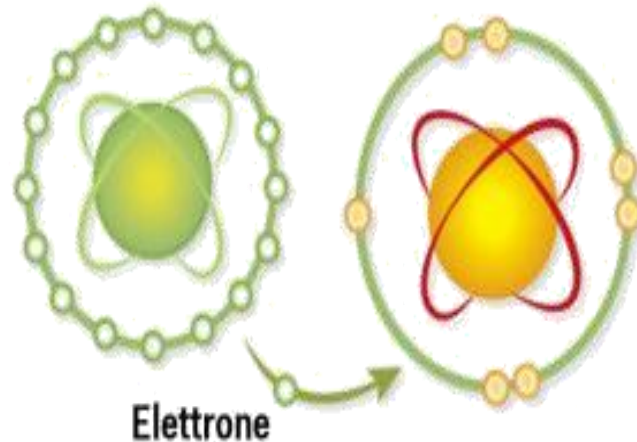
✓ Proteine

Studi recenti hanno dimostrato le **proprietà antinfiammatorie, antiossidanti e antitumorali di alcuni peptidi bioattivi** derivanti da **microalghe** con possibili future applicazioni nel campo farmaceutico (Ovando et al., 2016; Saha et al., 2016)



Antiossidante

Radicale libero



Potenziali clienti sul mercato



3^a età



Sportivi



Bambini



Vegetariani e vegani



Donne in menopausa




Donne in gravidanza ed allattamento

Fare sempre riferimento ad un medico e/o un nutrizionista

Journal of Applied Phycology (2019) 31:1077–1083
<https://doi.org/10.1007/s10811-018-1602-3>



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

Alberto Niccolai¹ • Emer Shannon² • Nissreen Abu-Ghannam² • Natascia Biondi¹ • Liliana Rodolfi^{1,3} • Mario R. Tredici¹ 

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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⁻¹ and lactic acid concentration reached 3.7 g L⁻¹. 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*

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DIT - School of Food Science, Dublin



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Biotechnologies for Environmental Management and Sustainable Agriculture

Natural Resources Management for Tropical Rural Development

Science and Management of Fauna and Environmental Resources

Agricultural Sciences and Technologies

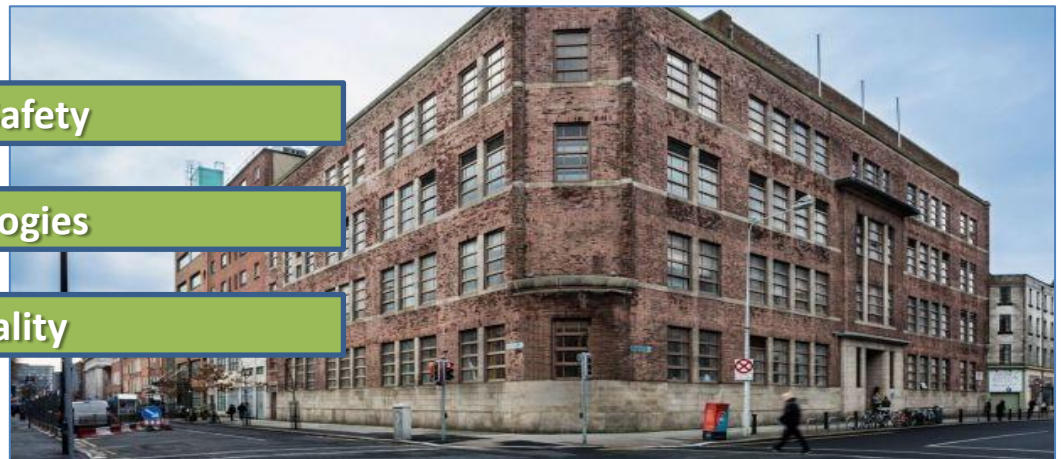


<https://www.dispaa.unifi.it/>

Environmental Health and Safety

Food Sciences and Technologies

Water Sciences and Air quality



<http://www.dit.ie/>

F&M Company

<http://www.femonline.it/>

Design and realisation of pilot and industrial photobioreactors



Selection of PUFA- and carotenoid-rich microalgae

Microalgae and cyanobacteria culture for food/feed/bioactives production



Selection of microalgae as promising feed for fish and mollusks

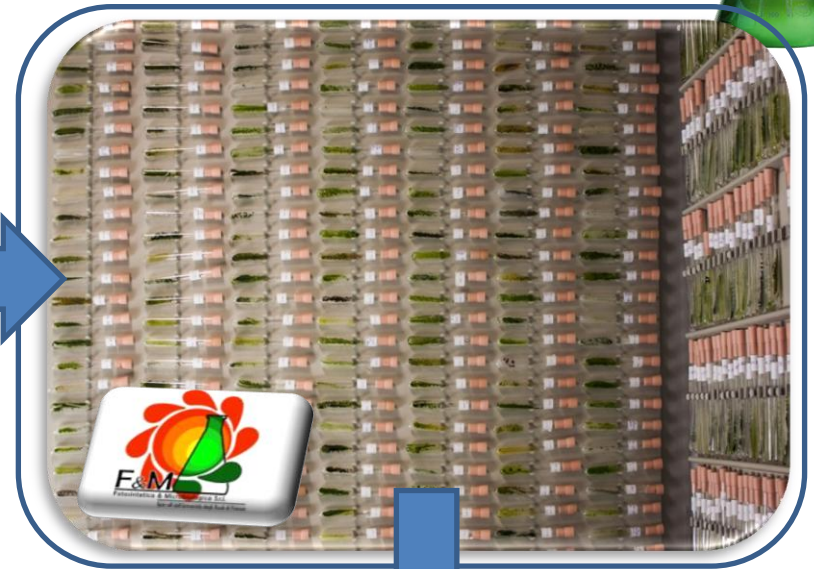
A. platensis F&M-C256 biomass production



**A. platensis F&M-C256
(spirulina)**



Does not require EU Novel
Food approval (Reg EC 258/97)



Bacterial inoculum preparation



Cruinn

Cruinn Diagnostics Limited



***L. plantarum* ATCC 8014**

Bacterial inoculum preparation



Cruinn

Cruinn Diagnostics Limited

1 mL of LAB 8014

25 mL



L. plantarum ATCC 8014



Bacterial inoculum preparation



Cruinn

Cruinn Diagnostics Limited

1 mL of LAB 8014

25 mL



L. plantarum ATCC 8014




37 °C for 24 h




Fermentation




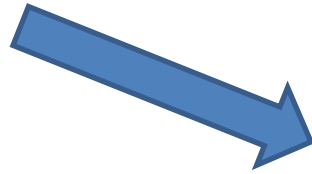
5 g



8% water content



**Lyophilised *A. platensis*
F&M-C256 biomass**



48 mL deionised water

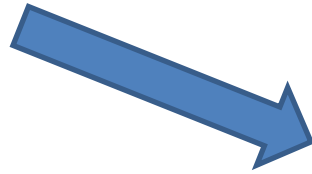
Fermentation



5 g

8% water content

**Lyophilised *A. platensis*
F&M-C256 biomass**

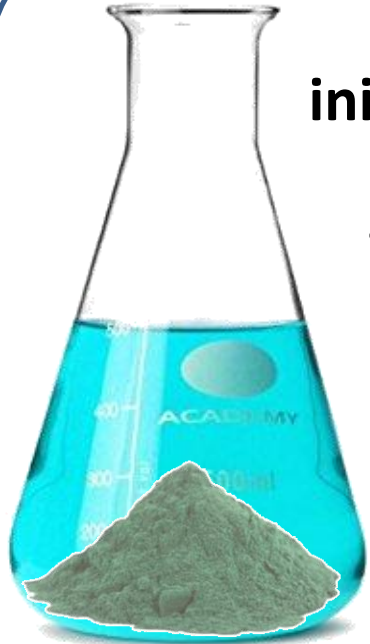


48 mL deionised water



**2 mL of
LAB 8014**

Fermentation



**initial *A. platensis* biomass
concentration of the
inoculated suspension**

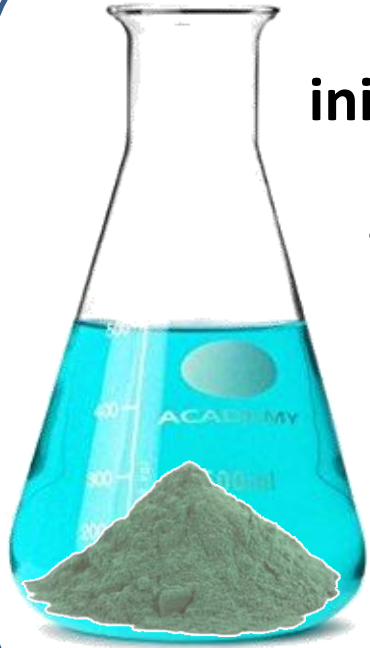


92 g dry weight L⁻¹

Fermentation



initial *A. platensis* biomass
concentration of the
inoculated suspension



92 g dry weight L⁻¹



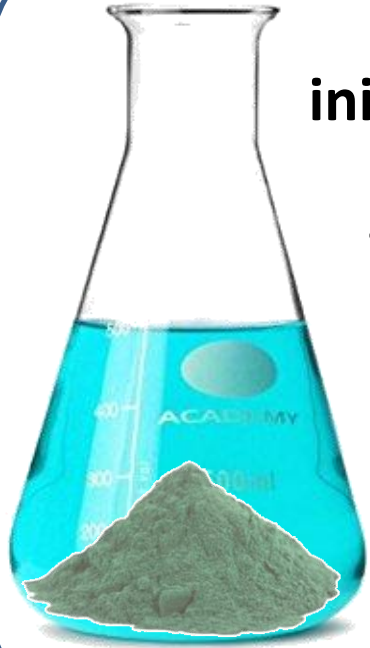
37 °C
100 rpm



Fermentation



initial *A. platensis* biomass
concentration of the
inoculated suspension



92 g dry weight L⁻¹

0 h

24 h

48 h

72 h



37 °C
100 rpm



Microbiological analyses



0 h



24 h



48 h



72 h



Microbiological analyses



0 h

24 h

48 h

72 h



Microbiological analyses

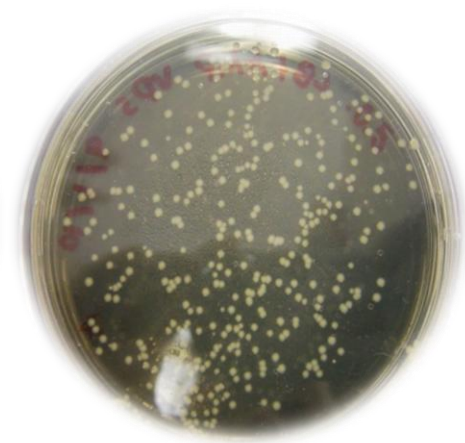


0 h

24 h

48 h

72 h



Lactic acid determination



0 h



24 h



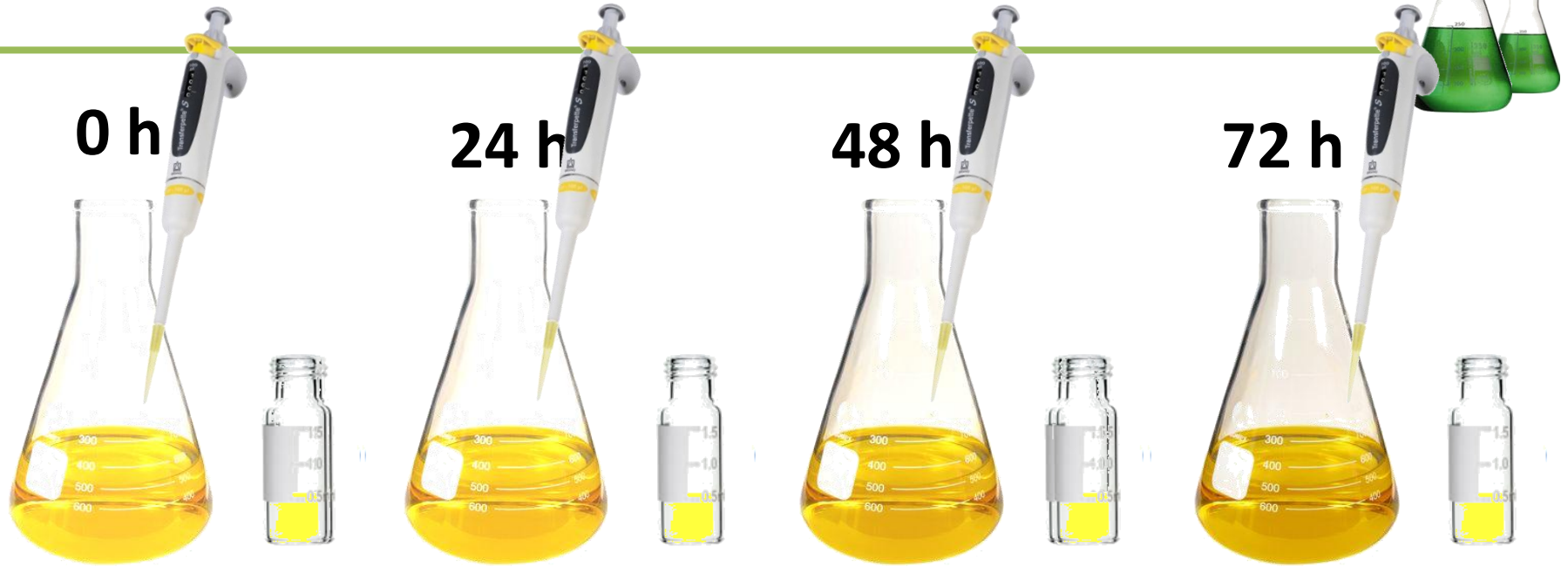
48 h



72 h



Lactic acid determination



Lactic acid determination



0 h



24 h



48 h



72 h



In vitro digestibility

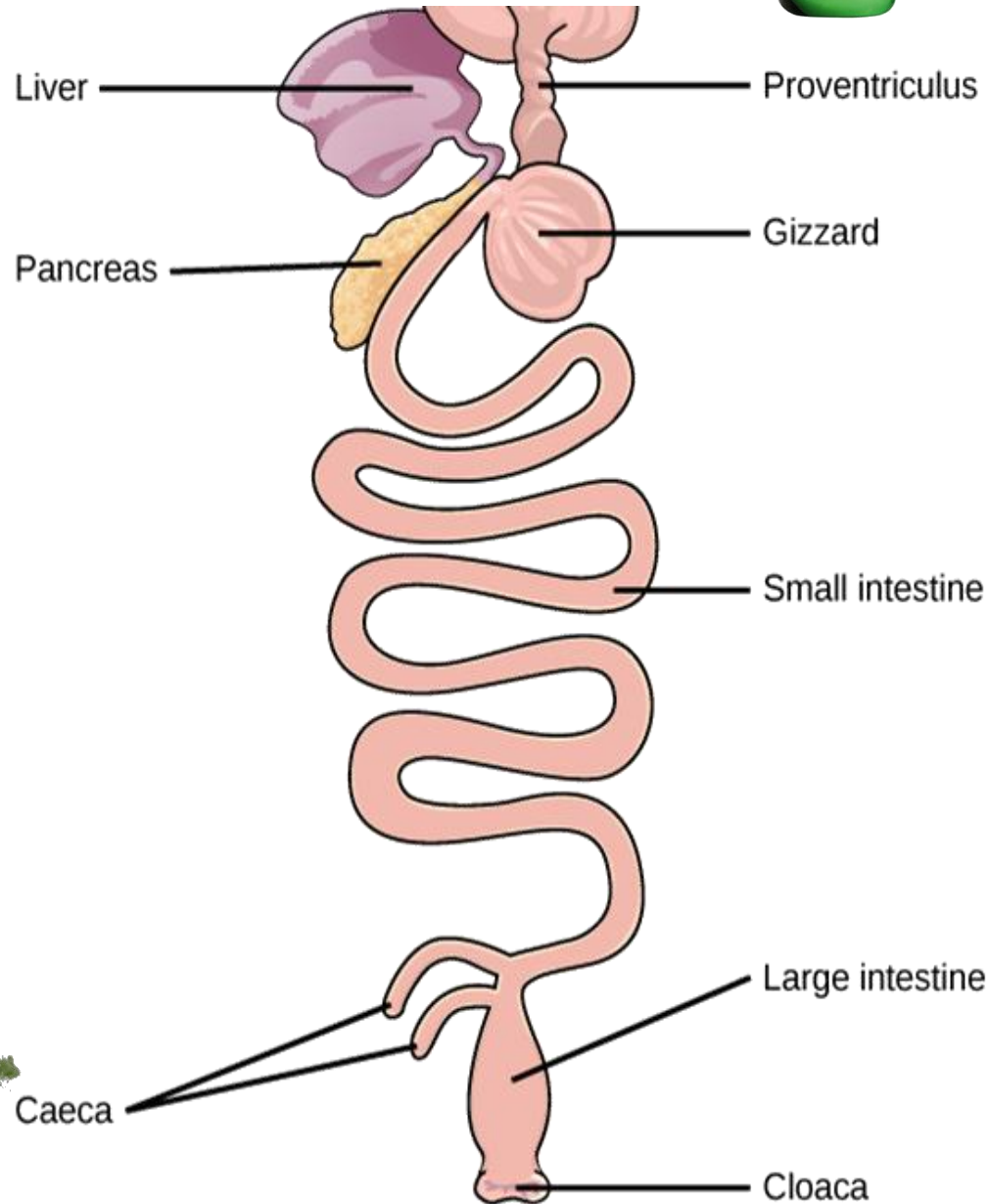
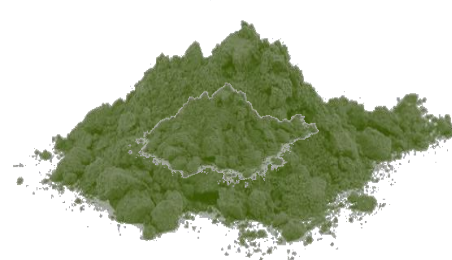


0 h

72 h



Boisen and Fernández (1997) method

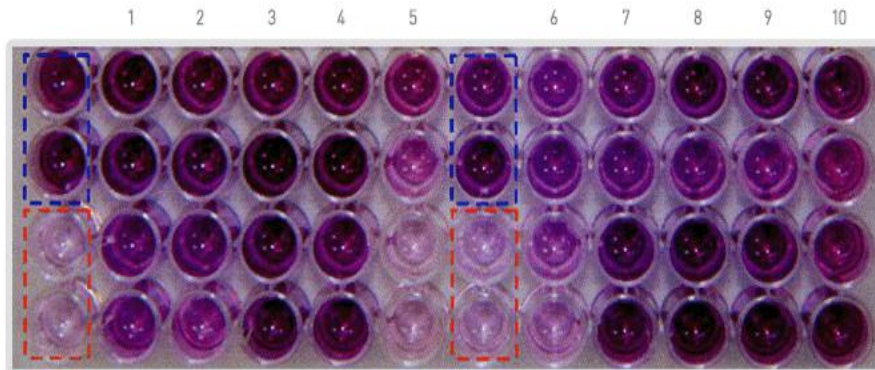
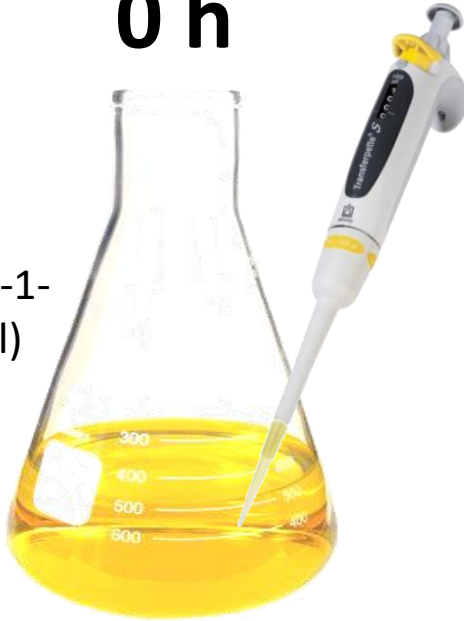


DPPH assay and total phenolic content determination



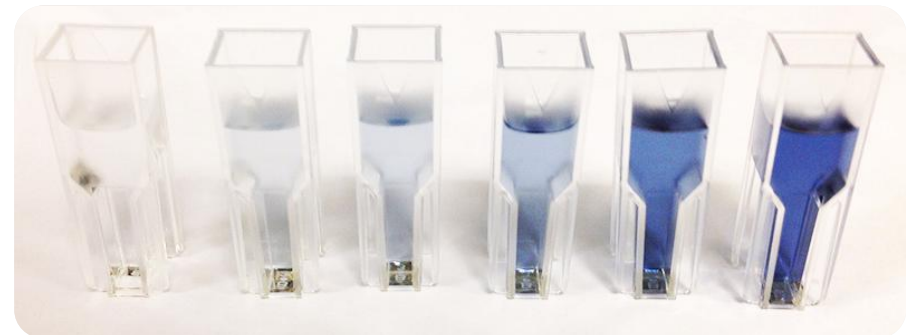
0 h

DPPH Assay
(2,2-diphenyl-1-picrylhydrazyl)

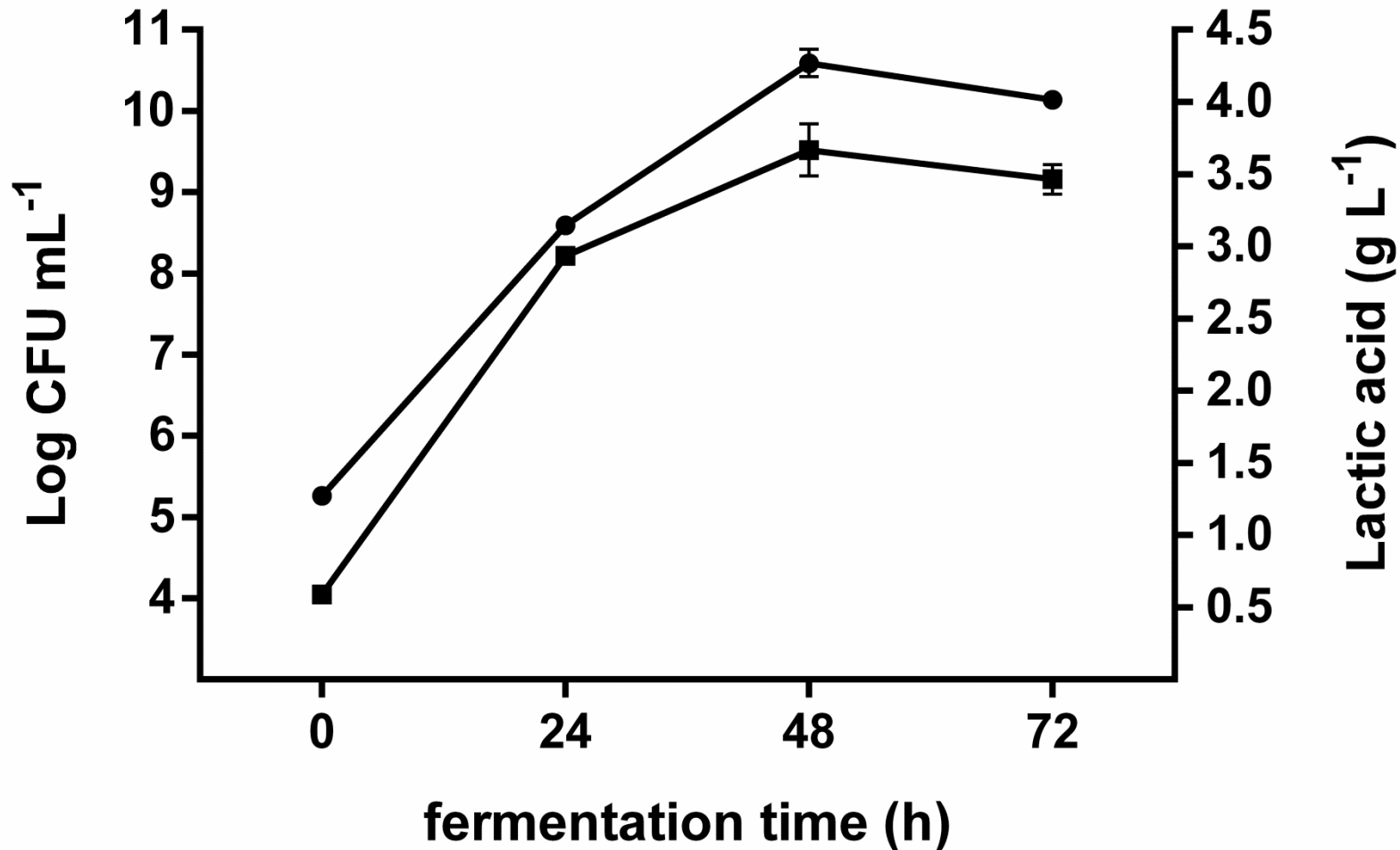


72 h

Folin-Ciocalteu Assay

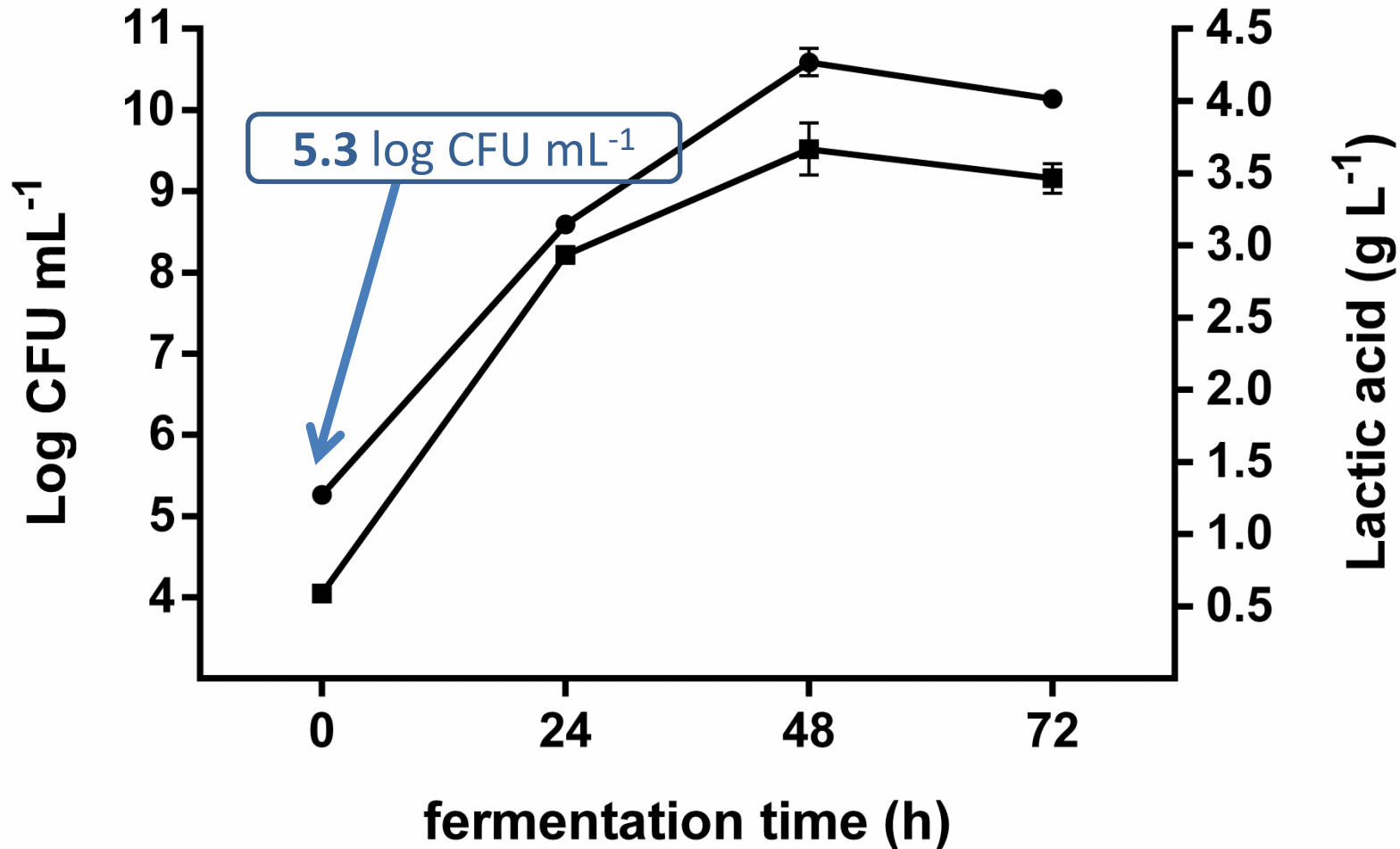


Suitability of *A. platensis* biomass for LAB growth and lactic acid fermentation



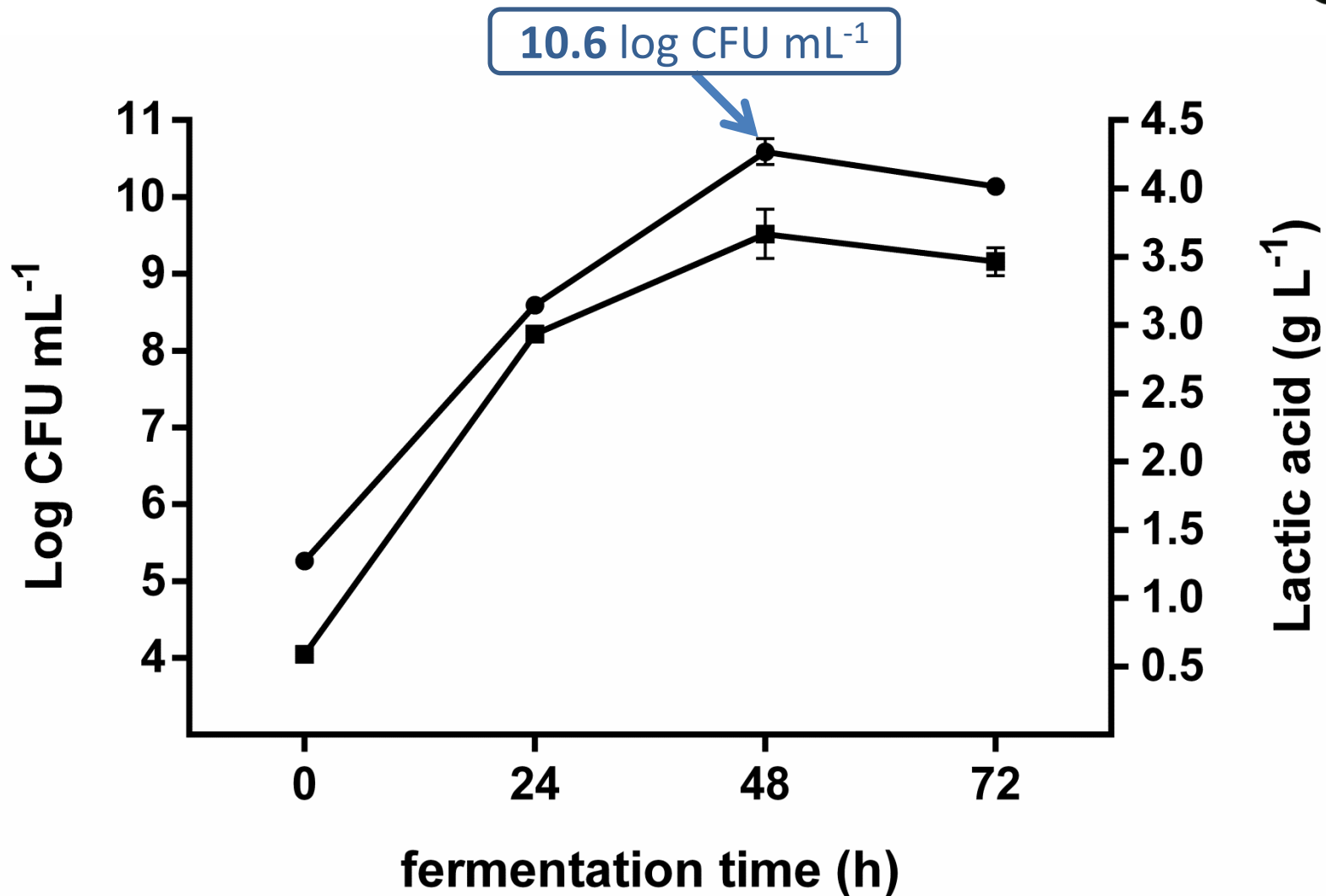
Growth curve (●) of and lactic acid production (■) by LAB 8014 in a broth containing *A. platensis* F&M-C256 biomass as the sole substrate. The amount of biomass at the start of the experiment (time zero) was 92 g (dry weight) L⁻¹. Values are expressed as means \pm SD.

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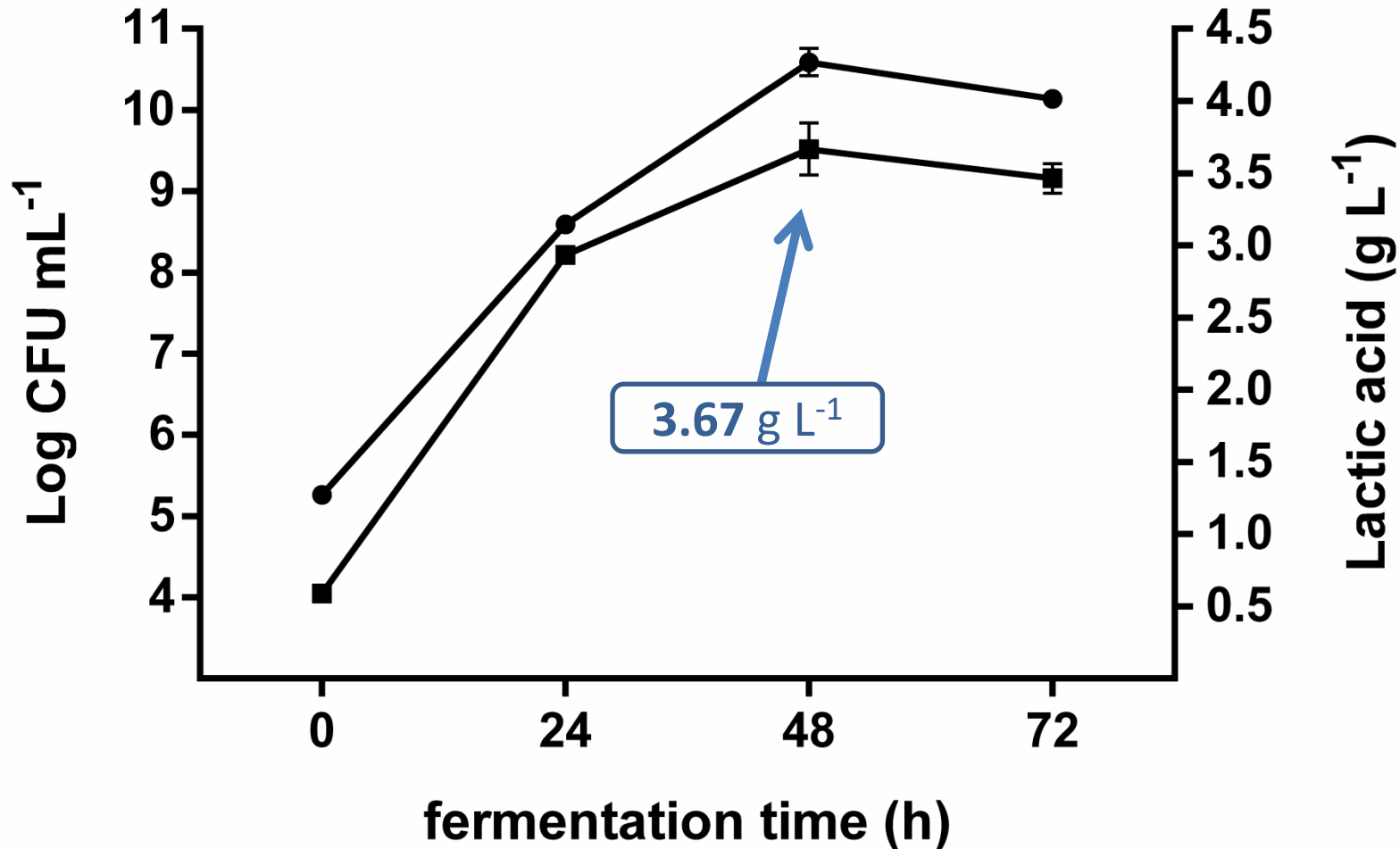
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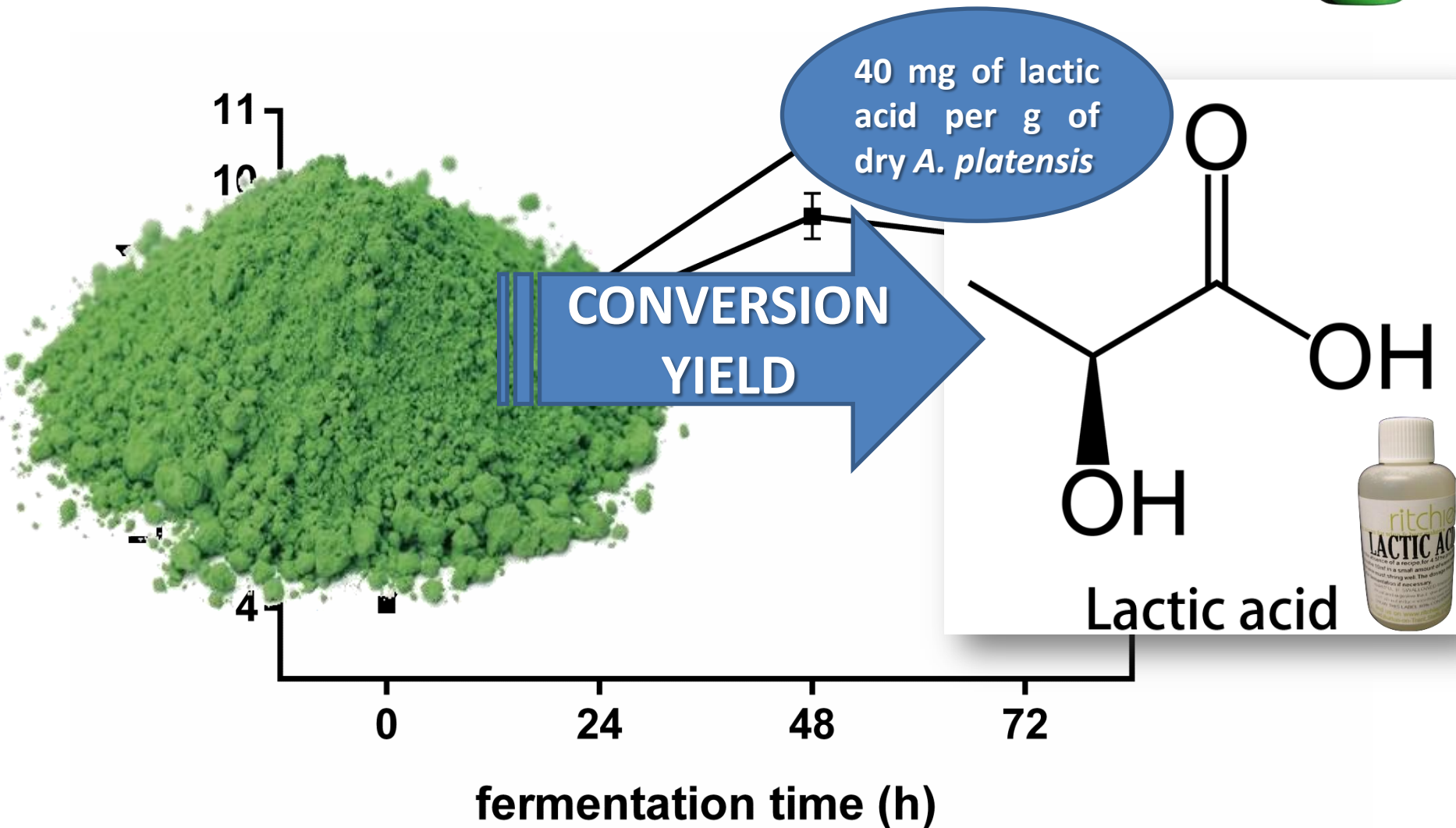
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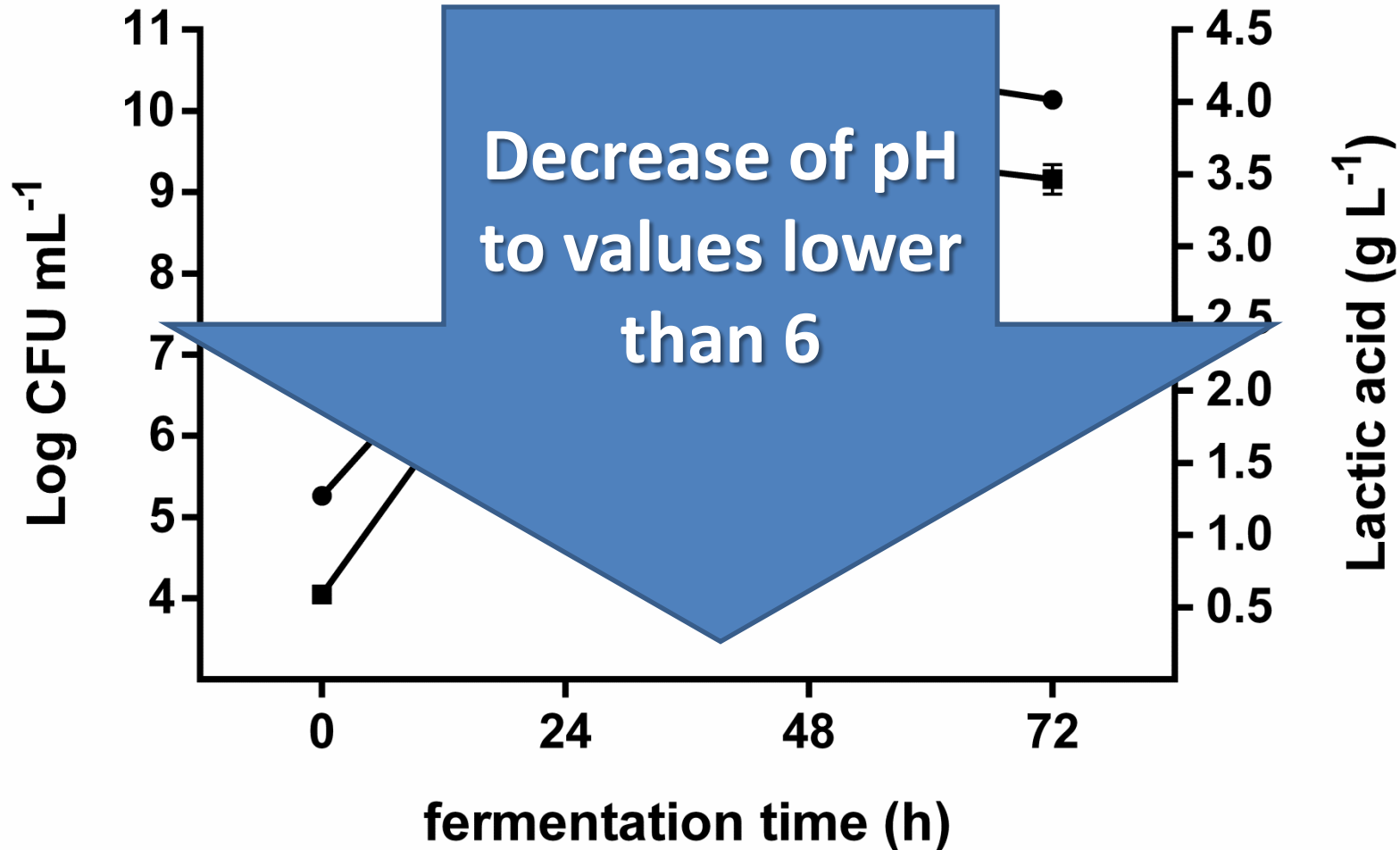
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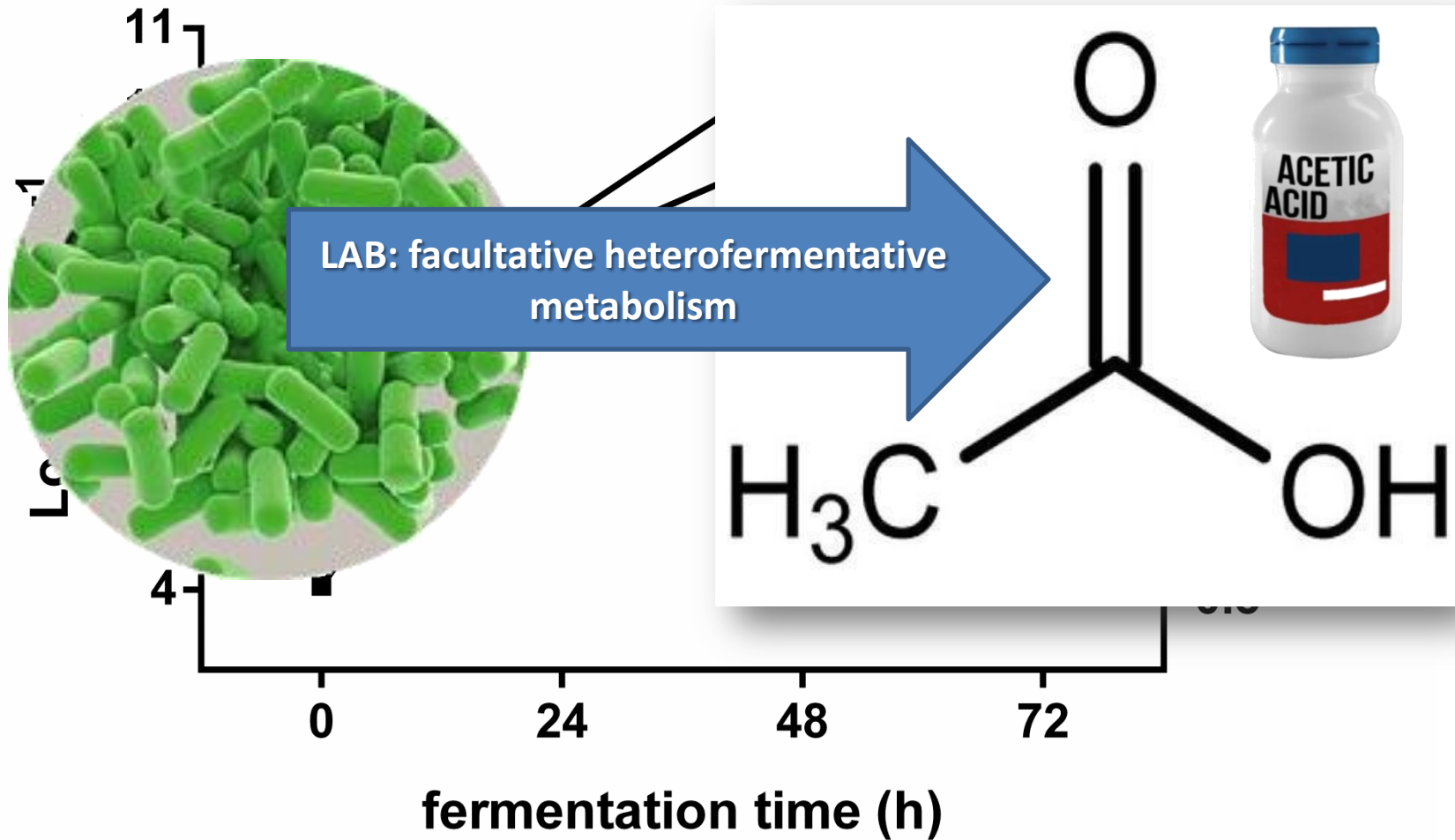
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Suitability of *A. platensis* biomass for LAB growth and lactic acid fermentation



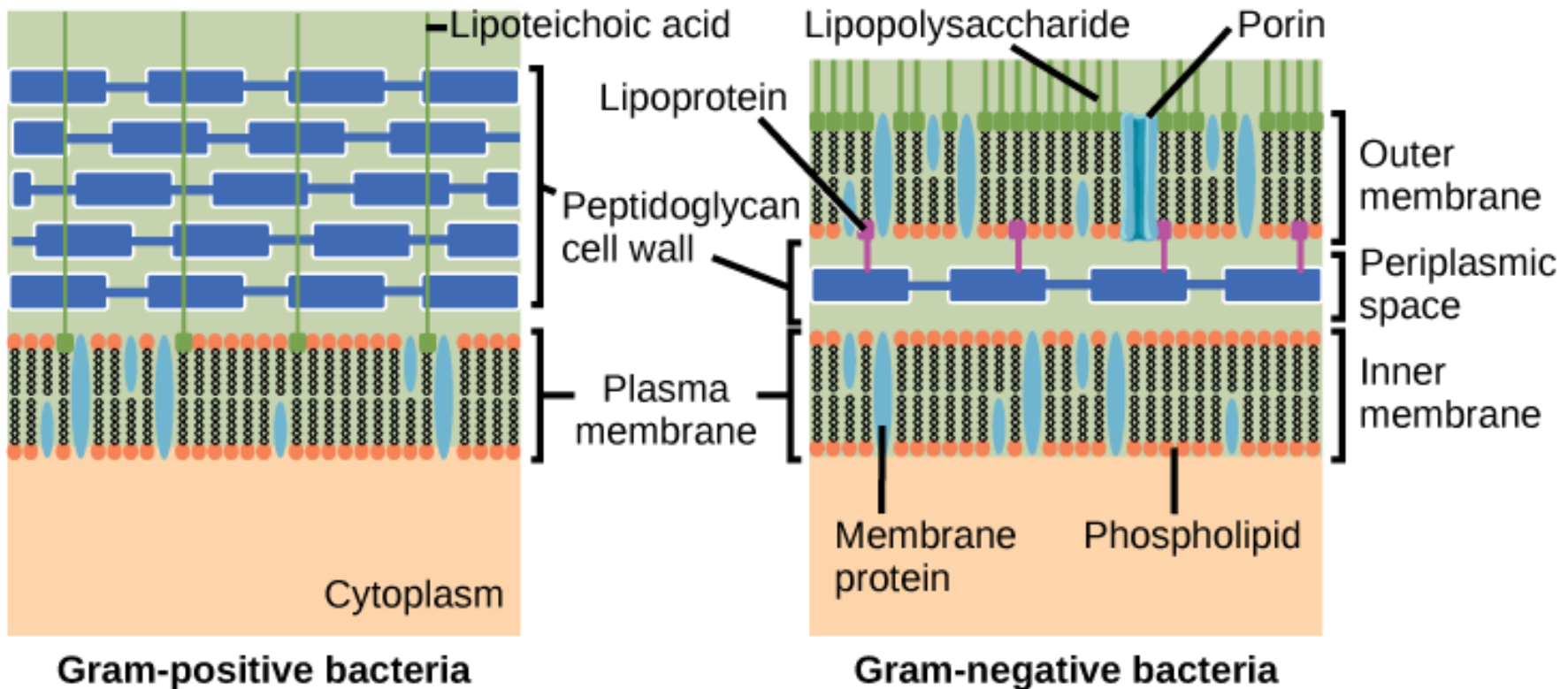
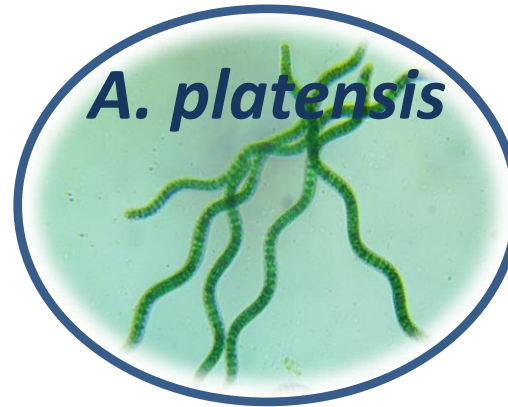
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Suitability of *A. platensis* biomass for LAB growth and lactic acid fermentation



Growth curve (●) of and lactic acid production (■) 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 zero) was 92 g (dry weight) L⁻¹. Values are expressed as means ± SD.

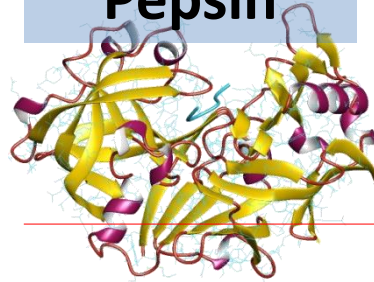
In vitro digestibility of fermented *A. platensis* biomass



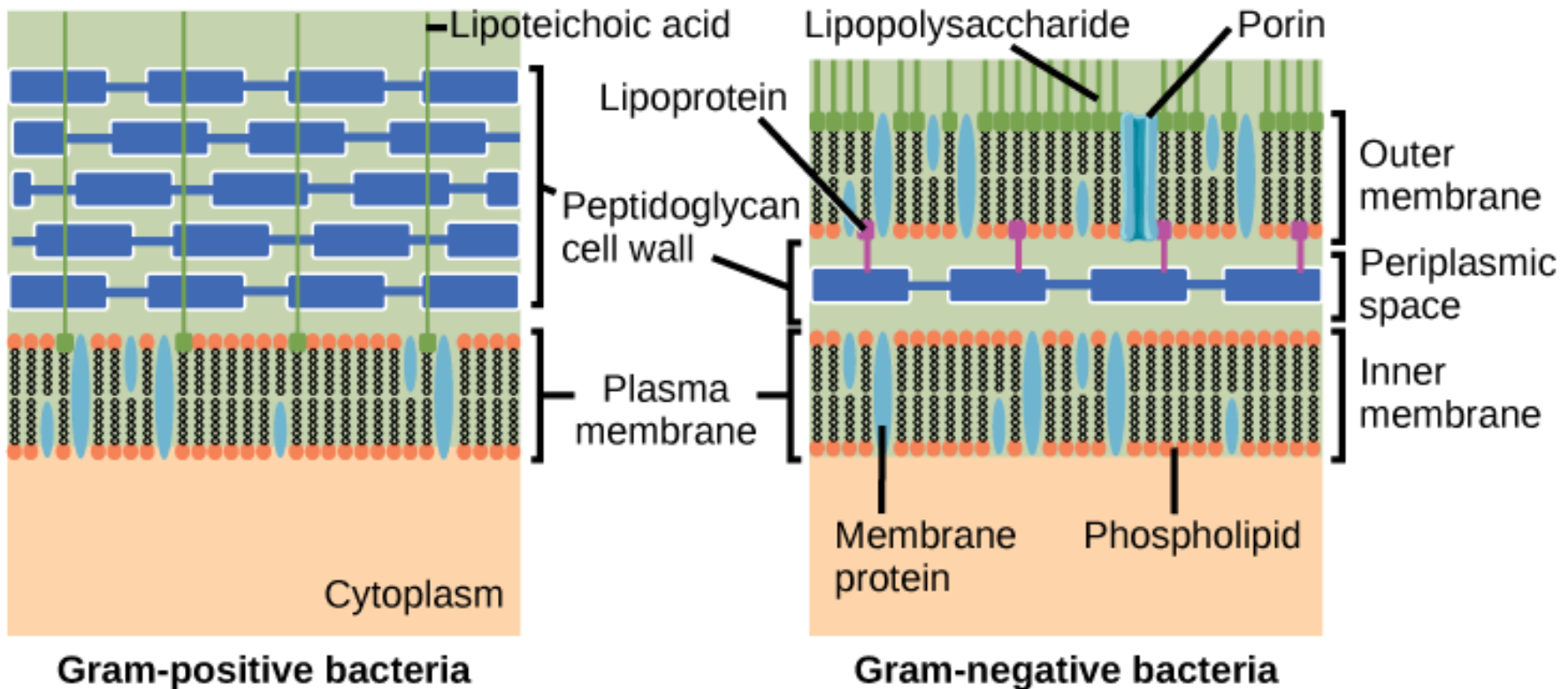
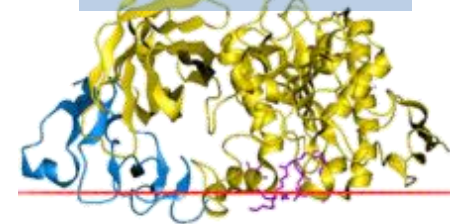
In vitro digestibility of fermented *A. platensis* biomass



Pepsin



Pancreatin

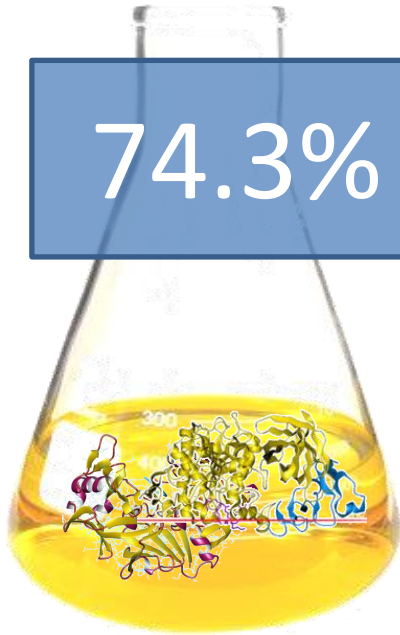


In vitro digestibility of fermented *A. platensis* biomass



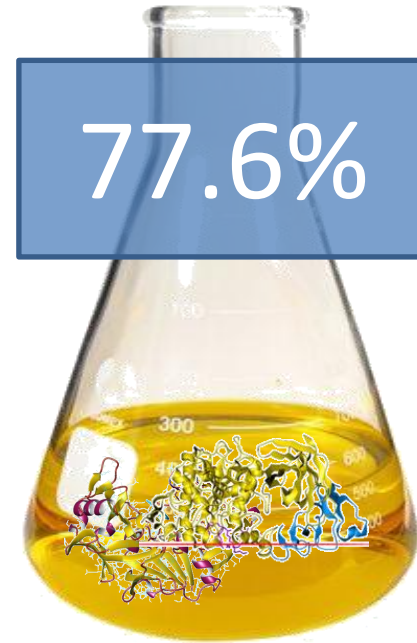
0 h

74.3%



72 h

77.6%



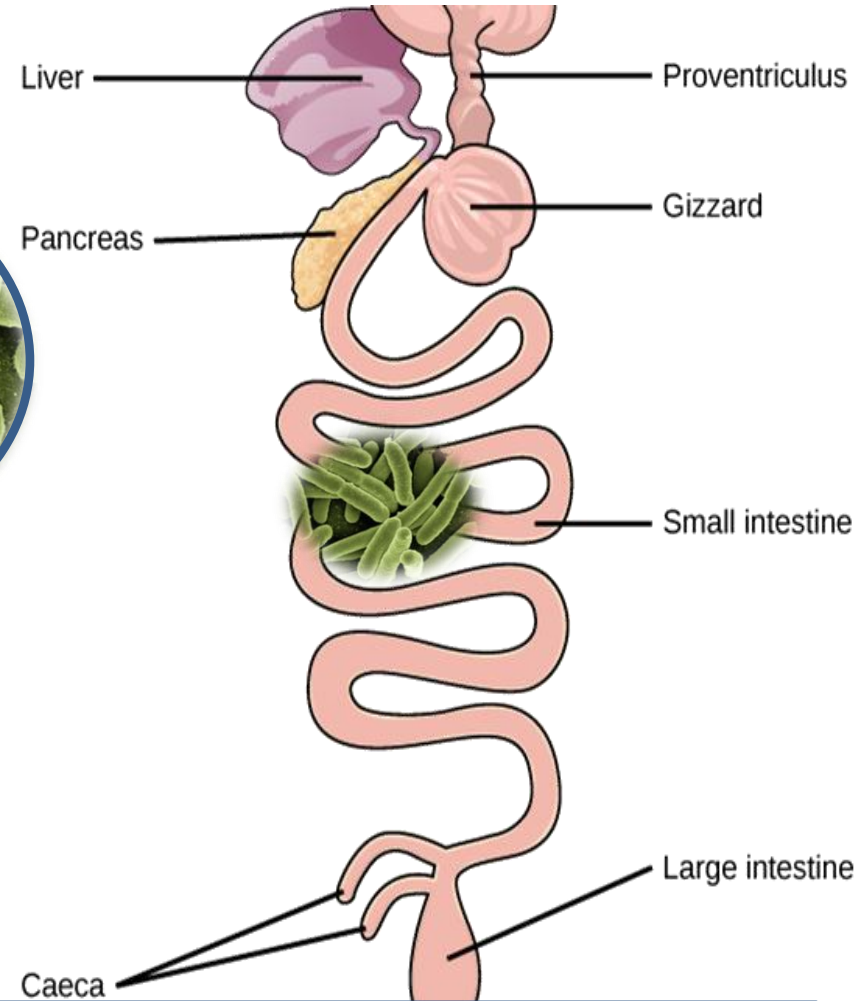
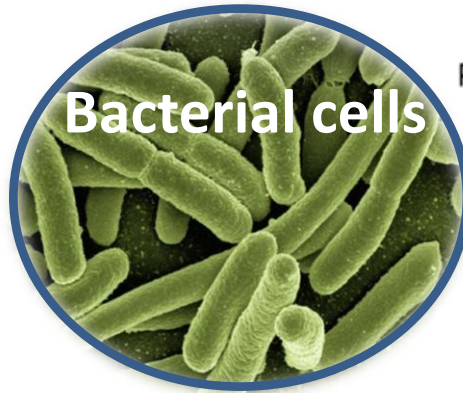
The small increase
observed (4.4%)
was not significant

In vitro digestibility (% dry matter) of lyophilised fermentation broth at the start and at the end of fermentation

In vitro digestibility of fermented *A. platensis* biomass



72 h



Low digestibility

Antioxidant capacity and phenolic content of fermented *A. platensis* biomass



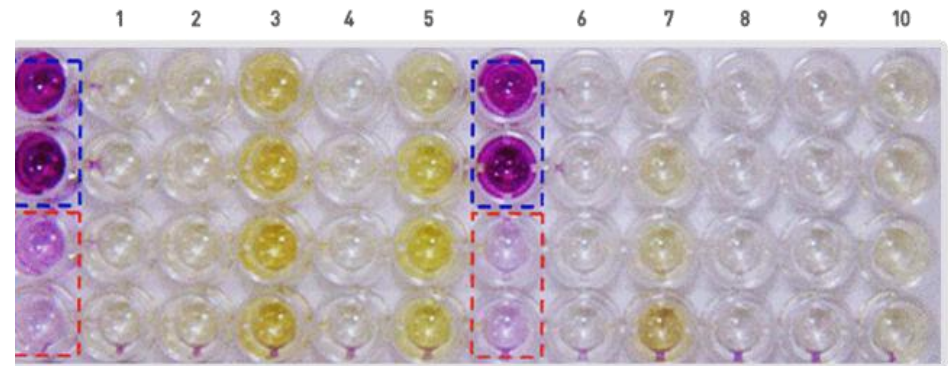
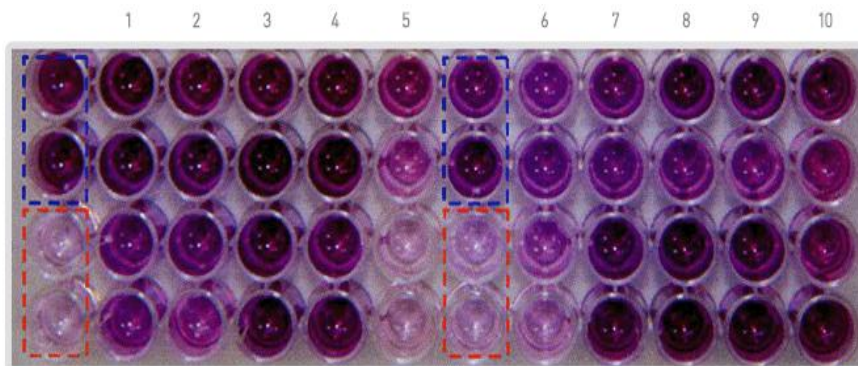
0 h

A 20.5% DPPH inhibition before fermentation



72 h

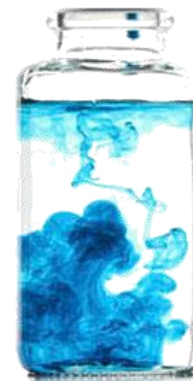
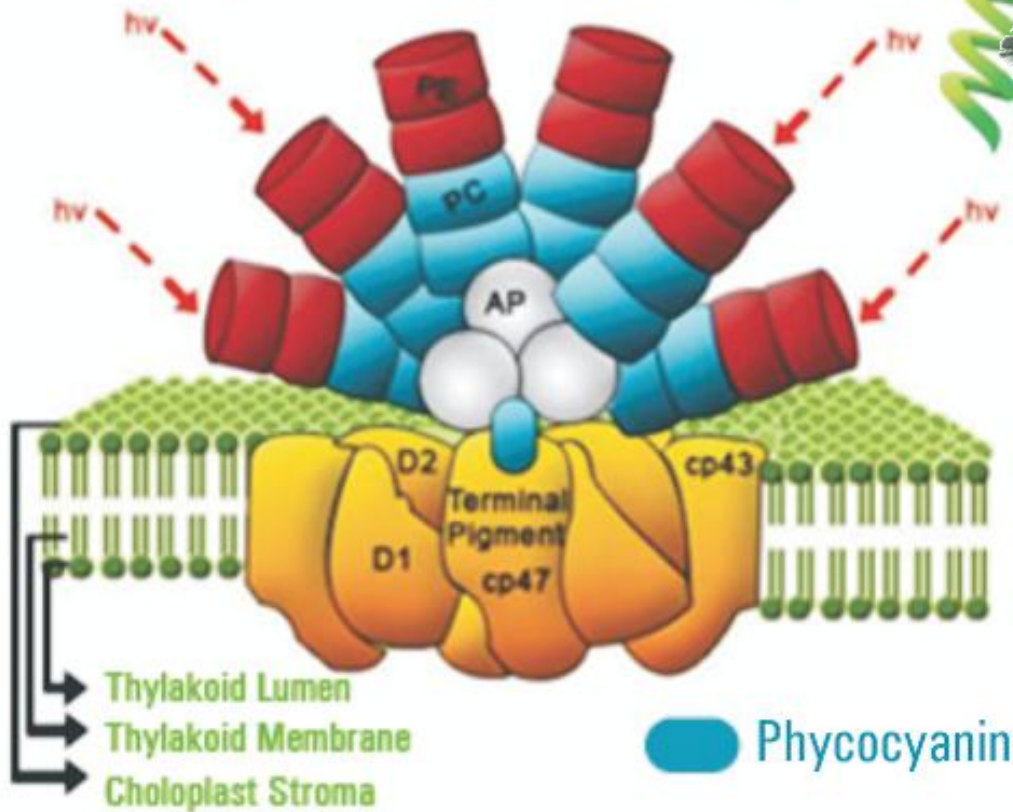
A 36.6% DPPH inhibition after fermentation



Antioxidant capacity and phenolic content of fermented *A. platensis* biomass



Structure of PHYCOCYANIN embedded inside Phycobilisome of *Arthrospira platensis*



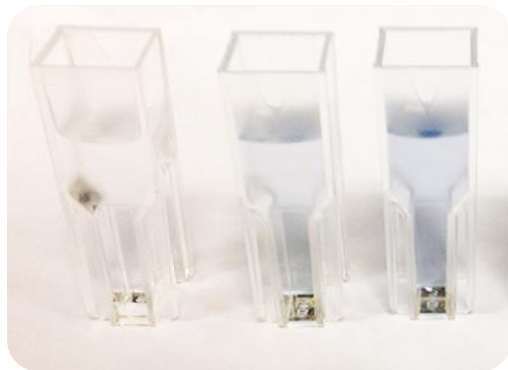
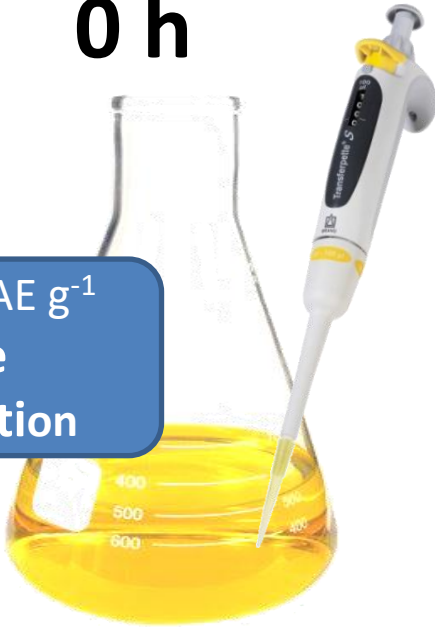
Phycocyanin contains an open chain tetrapyrrole chromophore, phycocyanobilin, which is covalently attached to an apoprotein

Antioxidant capacity and phenolic content of fermented *A. platensis* biomass



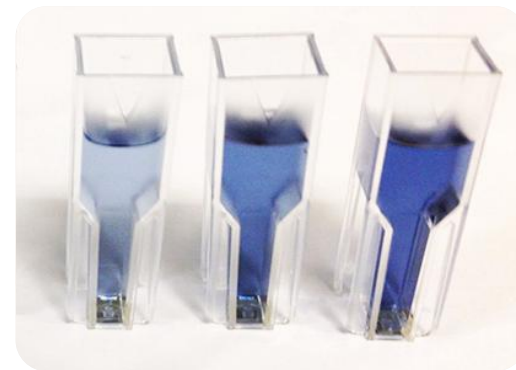
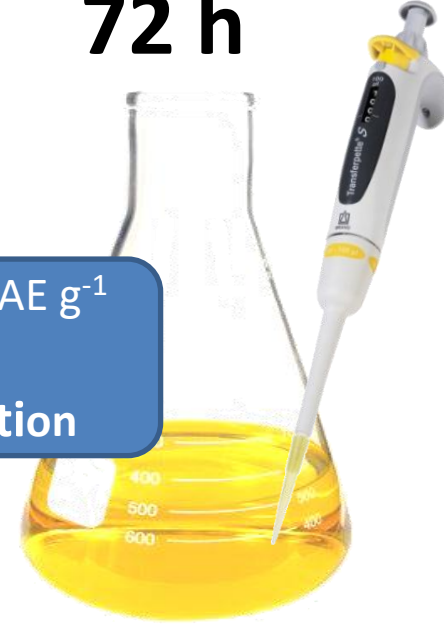
0 h

A 4.5 mg GAE g⁻¹
before
fermentation

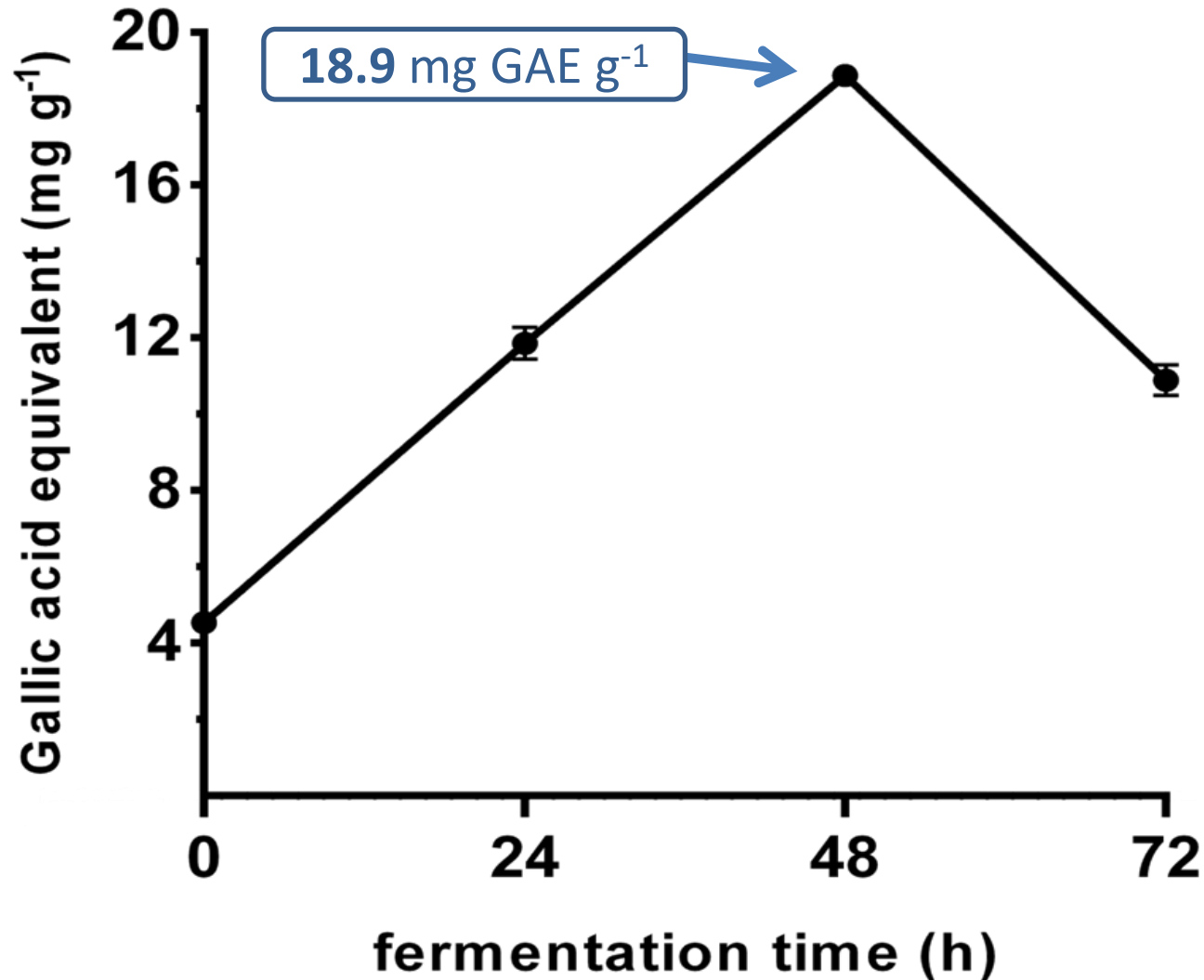


72 h

A 10.9 mg GAE g⁻¹
after
fermentation

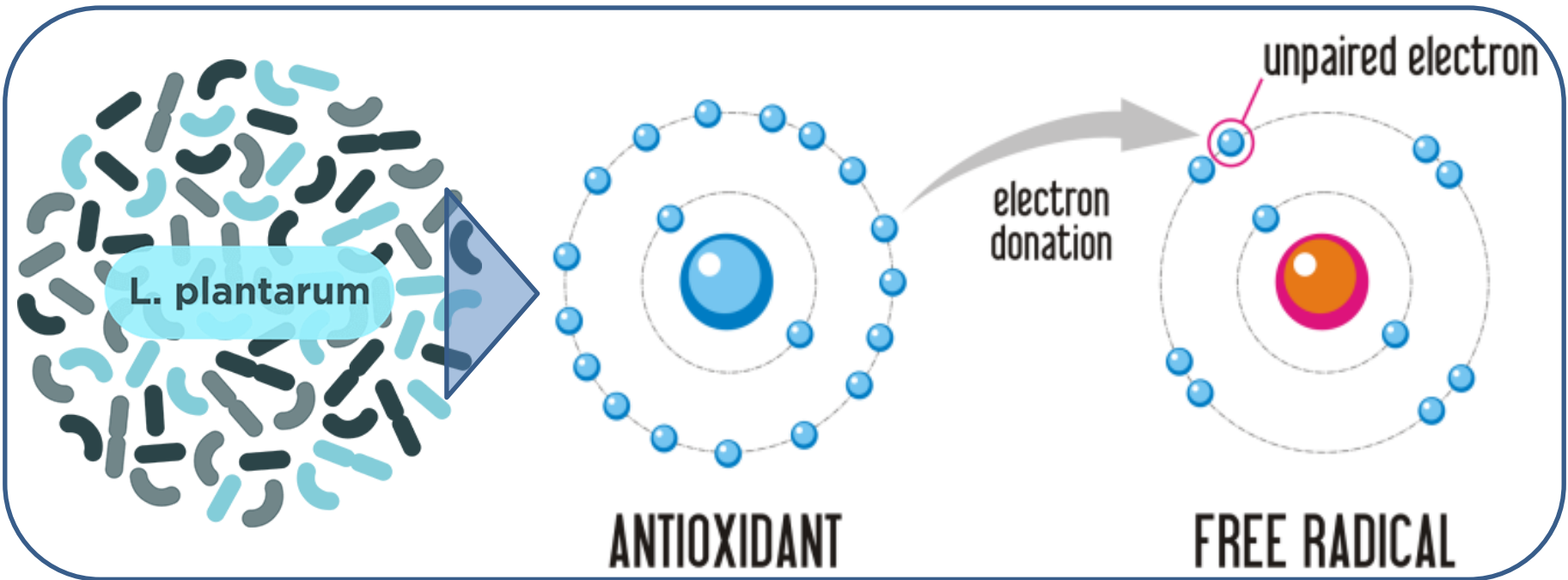


Antioxidant capacity and phenolic content of fermented *A. platensis* biomass



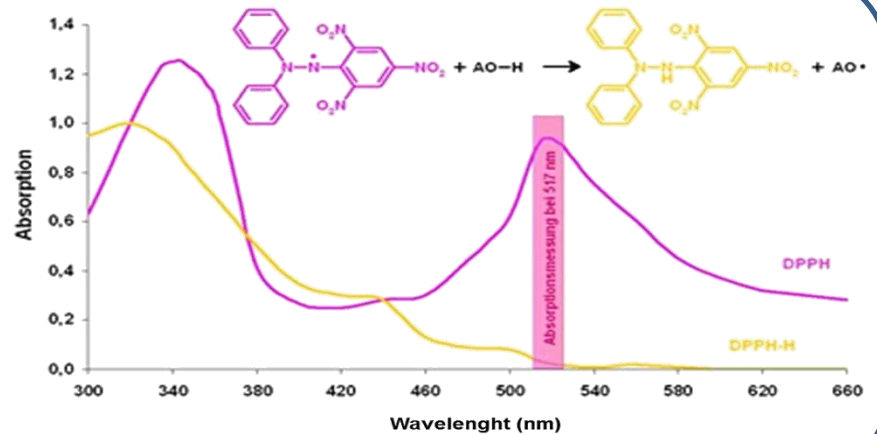
Total phenolic content, expressed as mg of gallic acid equivalent (GAE) per g of dry fermentation broth initially containing 92 g (dry weight) L⁻¹ of *A. platensis* biomass. Values are expressed as means \pm SD.

Antioxidant capacity and phenolic content of fermented *A. platensis* biomass



**L-3-(4-hydroxyphenyl)-
lactic acid**

**L-indole-3-
lactic acid**



Suzuki et al. (2013)

Concluding remarks



This study shows that *A. platensis* F&M-C256 is a **suitable growth substrate** for *Lactobacillus plantarum* ATCC 8014

Concluding remarks



This study shows that *A. platensis* F&M-C256 is a **suitable growth substrate** for *Lactobacillus plantarum* ATCC 8014



At the end of fermentation *L. plantarum* ATCC 8014 cells **constitute** more than **20% of the broth** dry weight

Concluding remarks



This study shows that *A. platensis* F&M-C256 is a **suitable growth substrate** for *Lactobacillus plantarum* ATCC 8014



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No significant enhancement in digestibility of *A. platensis* F&M-C256 biomass was observed following lactic acid fermentation

Concluding remarks



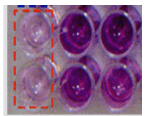
This study shows that *A. platensis* F&M-C256 is a **suitable growth substrate** for *Lactobacillus plantarum* ATCC 8014



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Antioxidant activity was strongly increased (of about 80%), mainly due to the rise in total phenolic content

Perspectives for the realization of functional products: YOGURT



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Effect of *Spirulina platensis* fortification on physicochemical, textural, antioxidant and sensory properties of yogurt during fermentation and storage



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ABSTRACT

Due to the high consumption rate of fermented milk products such as yogurt, the fortification of these products will effectively reduce diseases associated with nutritional deficiencies. In the present study, after incorporating *Spirulina* into yogurt at four different concentrations (0.25, 0.5, 0.75 and 1%), we studied its effect on the fermentation process, texture, nutraceutical and sensory characteristics of yogurt. The addition of 0.25% of *Spirulina* was significantly sufficient to accelerate the end of fermentation ($p < 0.05$) and conserve the textural properties and sensory acceptability of the final milk product. This treatment also exhibited significant better water holding capacity and lower whey syneresis during 28 days of storage. During this period, the colored yogurt showed negligible variations for the L^* , a^* and b^* indices, reflecting the strong stability of *Spirulina* color. Thanks to its high content in pigments, *Spirulina* considerably improve the antioxidant activity of the new formulated yogurt. Overall, it can be concluded that *Spirulina* can be used as a natural ingredient to develop a novel yogurt with high nutritional properties.

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Perspectives for the realization of functional products: YOGURT



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Yogurt greco con Spirulina



Lo **yogurt greco** è più compatto di quello tradizionale bianco e ha un sapore completamente diverso.

Lo yogurt è un derivato del latte che può essere di qualsiasi animale (capra, mucca e pecora) e anche di origine vegetale (come il latte di soia, di riso, di noci...)

perché deriva essenzialmente dalla fermentazione degli zuccheri, processo durante il quale il lattosio è trasformato in acido lattico che dona il tipico sapore acidulo allo yogurt.



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Perspectives for the realization of functional products: YOGURT



to improve the antioxidant potential of the product

to fortify textural and physicochemical properties

to enrich the product with probiotics with
a positive effect on sensorial properties

Conclusions

Fermentation by lactic acid bacteria is a **suitable technology to obtain innovative functional products** (such as **yogurt**) from ***A. platensis* F&M-C256**, which can provide to the consumer, besides the **highly nutritional components of spirulina** a **significant amount of probiotic *Lactobacillus* cells** conferring additional beneficial properties to the final product.



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Many thanks for your attention



Per domande e possibili collaborazioni

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