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Stimulative effect of lactic acid bacteria in the growth of the microalgae *Isochrysis galbana*

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ABSTRACT

Objective: To study the effect of species of lactic acid bacteria (LAB) from telluric origin on the growth of the microalgae *Isochrysis galbana* (*I. galbana*) in small and medium volume flasks.

Methods: In the first experiment, 7 LAB species [*Carnobacterium piscicola*, *Lactobacillus brevis*, *Lactobacillus casei* ssp. *casei, Lactobacillus helveticus, Lactococcus lactis* spp. *lactis, Leuconostoc mesenteroides* spp. *mesenteroides* (*L. mesenteroides* spp. *mesenteroides*) and *Pediococcus acidilactici* (*P. acidilactici*)] were inoculated in 250 mL flasks containing microalgae *I. galbana* (10^6 cells/mL). After fitting the growth data to two mathematical models, two LAB strains (*L. mesenteroides* spp. *mesenteroides* and *P. acidilactici*) were selected for the second experiment in which those strains were inoculated in medium size (5 L) volume cultures of *I. galbana* (1.2×10^5–1.5×10^5 cells/mL). The bacterial load in cultures from the first experiment was analyzed by plating on marine agar, MRS agar and thiosulfate citrate bile saltssucrose media.

Results: All strains of LAB tested enhanced the growth rate and the final biomass yield of *I. galbana* cultures, even in the absence of nutrients in the media. The best overall results and the maximal final cell densities in small flasks were achieved with strains *L. mesenteroides* spp. *mesenteroides* and *P. acidilactici*, respectively. These two strains also stimulated the growth (40% and 16% with respect to controls) of *I. galbana* in medium size volumes. For most strains, CFU values of LAB remained stable (10^5–10^8 CFU/mL) for at least 4 days. A high variability was observed in bacteria strains among treatments, with *Pseudomonas* and *Moraxella* being the most abundant bacteria.

Conclusions: The results of present study showed that the growth of *I. galbana* in both small and medium size volumes was enhanced by LAB, both in the absence and the presence of nutrients in the culture. The highest final biomass was achieved by adding *P. acidilactici*, whereas *Carnobacterium piscicola* and *L. mesenteroides* spp. *mesenteroides* provided maximal growth rates. The former also showed an inhibitory effect on *Moraxella*.

1. Introduction

Microalgae are used as food for live prey (rotifers, *Artemia*), larvae and adults of fish, molluscs and crustaceans. Algae can produce compounds that promote or inhibit bacterial growth and bacteria have a negative or positive effect on microalgae cultures[1-3]. Some bacteria strains have been used as probiotics or prebiotics in the rearing of aquatic organisms to prevent bacterial diseases or to improve growth and survival[3-10]. In addition, it has been demonstrated that the manipulation of the microbial community present in a rearing system may also contribute to the enhancement of microalgae and live feed cultures, including the reduction in potentially pathogenic bacterial strains[11-13]. The bacterial load of rearing systems can be moderately manipulated by adding beneficial bacteria to the culture media or by bioencapsulating the bacteria in live prey[10].

The selection and application of bacteria as probiotics in microalgae cultures requires to discard potential inhibiting or detrimental effects in the cultures. It is known that bacteria can enhance or suppress the growth of certain microalgae species[1,14-24]. In most studies focussed on bacteria and microalgae interactions, the growth of microalgae has been investigated using marine bacteria but the promoting effect of telluric bacteria strains has been only exceptionally tested[25].

In the present study, the effect of seven strains of lactic acid bacteria (LAB) from terrestrial origin on the performance of

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Isochrysis galbana (*I. galbana*) cultures was assayed in small and medium size volumes in order to discard the inhibitory effects. In a previous study, we demonstrated that those strains promote the growth of the rotifer *Brachionus plicatilis* in culture[12].

2. Materials and methods

2.1. Preparation of LAB

Cultures of seven species of LAB (Table 1) were maintained as frozen stocks at -50 ˚C in powdered skimmed milk suspensions containing 25% (v/v) of glycerol[26]. Batch cultures were carried out in a rotary agitator (200 r/min) at 30 ˚C in 250 mL Erlenmeyer flasks with the optimum volumes of MRS agar media (Man, Rogosa, Sharpe, Pronadisa S.A.)[26]. Bacteria inocula were prepared as previously reported[12]. The bacterial biomass of each culture was harvested and delivered to the cultures of microalgae eliminating all traces of post-incubation medium.

Table 1
LAB strains tested in *I. galbana* cultures.

Species	Key	Origin
C. piscicola	Cb 1.01	CECT 4020
L. brevis	Lb 2.01	CECT 216
Lactobacillus casei ssp. *casei*	Lb 3.04	CECT 4043
Lactobacillus helveticus	Lb 6.04	CECT 541
Lactococcus lactis spp. *lactis*	Lc 1.04	CECT 539
L. mesenteroides spp. *mesenteroides*	Ln 3.07	CECT 4046
P. acidilactici	Pc 1.02	NRRL B-5627

C. piscicola: *Carnobacterium piscicola*; *L. brevis*: *Lactobacillus brevis*; *L. mesenteroides* spp. *mesenteroides*: *Leuconostoc mesenteroides* spp. *mesenteroides*; *P. acidilactici*: *Pediococcus acidilactici*; CECT: Spanish Type Culture Collection. NRRL: Northern Regional Research Laboratory (Peoria, Illinois, USA).

2.2. Culture of I. galbana

2.2.1. Experiment 1: culture in 250 mL flasks

Small volume cultures of *I. galbana* were carried out in 250 mL of 8 Erlenmeyer flasks containing 100 mL of filtered (0.2 μm) and sterilised seawater, and a mixture of Walne medium[27]. The initial microalgae inocula were established at 10^6 cells/mL. One flask remained as control whereas the other seven flasks were inoculated (0.5 g/L) with one of the following LAB (Table 1): *C. piscicola*, *L. brevis*, *Lactobacillus casei* ssp. *casei, Lactobacillus helveticus, Lactococcus lactis* spp. *lactis, L. mesenteroides* spp. *mesenteroides* and *P. acidilactici*. Due to their higher capacity of bacteriocins production[28], the initial densities for Pc 1.02 and Lc 1.04 were 0.225 and 0.2 g/L, respectively.

The cultures were maintained for 9 days (until the advanced stationary phase) at a controlled temperature of (22 ± 1) ˚C and a light intensity of about 10000 μmol·m^{-2}·s^{-1}, provided by day light fluorescent bulbs. The flasks were not aerated but they were shaken twice daily to avoid the sedimentation of algal cells. Cell densities of *Isochrysis* were daily recorded using a Thoma Neubauer chamber under a microscope (Nikon). The experiment was run by duplicate.

2.2.2. Experiment 2: culture in 5 L flasks

The big volume cultures were performed in 5 L flatbottom flasks at (23 ± 1) ˚C. Two groups of 3 flasks containing 4 L of sterile seawater were used. *I. galbana* was inoculated to each flask at a density of 1.2×10^5–1.5×10^5 cells/mL. In each group, one flask remained as control whereas the other two were inoculated with 0.5 and 0.225 g/L of *L. mesenteroides* spp. *mesenteroides* (Ln 3.07) and *P. acidilactici* (Pc 1.02), respectively. Walne media was only added to flasks in one group. All flasks were conveniently aerated. Cell densities of *Isochrysis* were daily recorded.

2.3. Fitting of experimental data and numerical methods

Microalgae cell numbers were counted daily using a Thoma's chamber. The growth of the microalgae was evaluated adjusting the original data to the following mathematical models:

1: Logistic model[29].

$$N = \frac{K}{1 + e^{c - r_m t}}$$

$$c = \mathrm{In}\left(\frac{K}{N_0} - 1\right) \quad [a]$$

2: Modified model of Gompertz[30].

$$\mathrm{In}\frac{N}{N_0} A \times \exp\left[-\exp\left(\frac{\mu_m \times \exp(1) \times (\lambda - t)}{A} + 1\right)\right] \quad [b]$$

where, N: Cell density (million cells/mL); N_0: Initial density; t: Time (days); K: Maximum population at infinite time (million cells/mL); r_m: Maximum specific growth rate (days^{-1}); μ_m: Maximum specific growth rate (days^{-1}); A: Coefficient of maximum population at infinite time (dimensionless); λ: Lag phase (days); N_m: Maximum population at infinite time (million cells/mL).

These models had some interesting characteristics; the clear pseudo-kinetic structure and the significance of equation parameters [a] and the capacity of equation parameters [b] described all the phases of the growth (lag phase, maximum rate and maximum growth or stationary phase). On the contrary, the structure of equation [b] was more complex and used the deceitful resort of the logarithmic normalization.

Fitting procedures and parametric estimations from the experimental results were performed using a non-linear least-squares (quasi-Newton) method provided by the macro Solver of the Microsoft excel XP spreadsheet. The sum of quadratic differences between observed and model-predicted values was minimised.

2.4. Bacteria load

In order to determine the effect of LAB treatments on the bacterial population associated to *I. galbana*, bacteriological studies were performed after 0, 1, 4, 6 and 8 days post flasks inoculation. Samples were taken from each experimental flask and 100 μL of each of serially dilutions were plated on marine agar (MA), on a selective medium for LAB (MRS agar) (Pronadisa) and on thiosulphate citrate bile saltssucrose medium (Panreac Química S.A). Plates were incubated for 48–72 h at room temperature (22 ˚C). Subsequently, the colonies were counted and the amount of bacteria was calculated (CFU/mL). Dominant colony types on plates were visually selected on

the basis of different colony appearance and abundance. The selected colonies from each plate were isolated and re-streaked on fresh MA to ensure purity. For long-term preservation, the cultures were frozen at -80 °C in tryptone soy broth containing 1% NaCl (tryptone soy broth-1) and 15% glycerol (v/v).

Pure cultures of the bacterial strains were subjected to standard morphological, physiological and biochemical plate and tube tests including Gram-character[30], oxidase test, morphology, motility, sensitivity against O/129 and growth on thiosulfate citrate bile saltssucrose.

3. Results

The delivery of LAB into small volume flasks (experiment 1) resulted in a significant increase in the growth rates of *I. galbana* compared to controls (Figures 1 and 2). The maximal cell densities achieved in control flasks were 4.3×10^6 cells/mL whereas the average density in challenged flasks was about 7×10^6 cells/mL. The highest cell densities were attained with the addition of Lb 3.04, Lc 1.04 and Pc 1.02 strains. A noticeable increase was also observed in r_m for Ln 3.07 and Cb 1.01. A massive turbidity appeared in the culture media after the addition of Lb 3.04 and Lc 1.04. In the former, the culture collapsed at Day 5 in one of the replicates. In the other replicate, the disappearance of turbidity after Day 5 promoted a huge increase in cell density. A similar fact was also noticed for Lc 1.04. Consequently, those data were excluded in the adjustment of experimental data to the fitting equation models [a] and [b] (Figures 1 and 2).

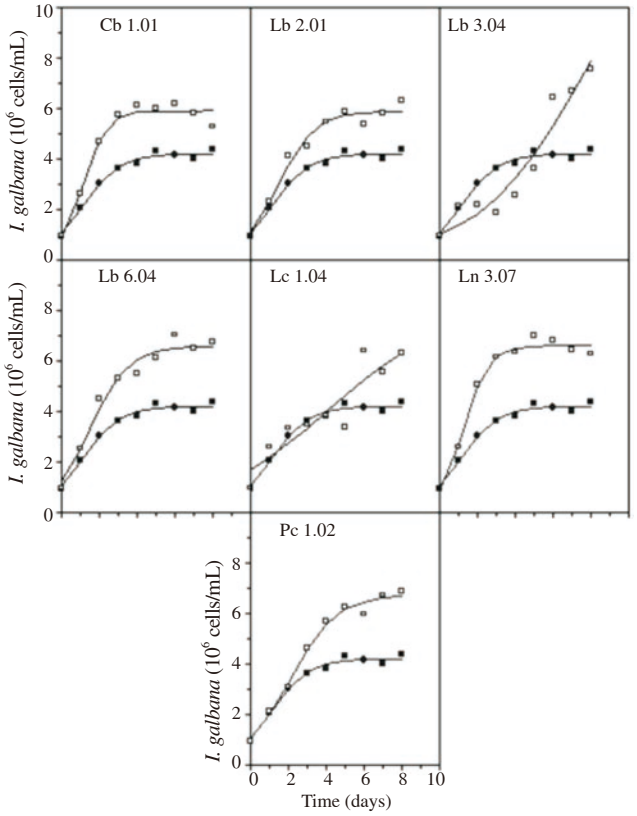

Figure 1. Effect of the addition of LAB on the growth of *I. galbana* in Erlenmeyer flasks (250 mL) (experiment 1).
White symbols: Addition; Black symbols: Control. Data fitted to equation [a]. Initial concentrations of LAB were Pc 1.02 = 0.225, Lc 1.04 = 0.200 and others = 0.500 g/L.

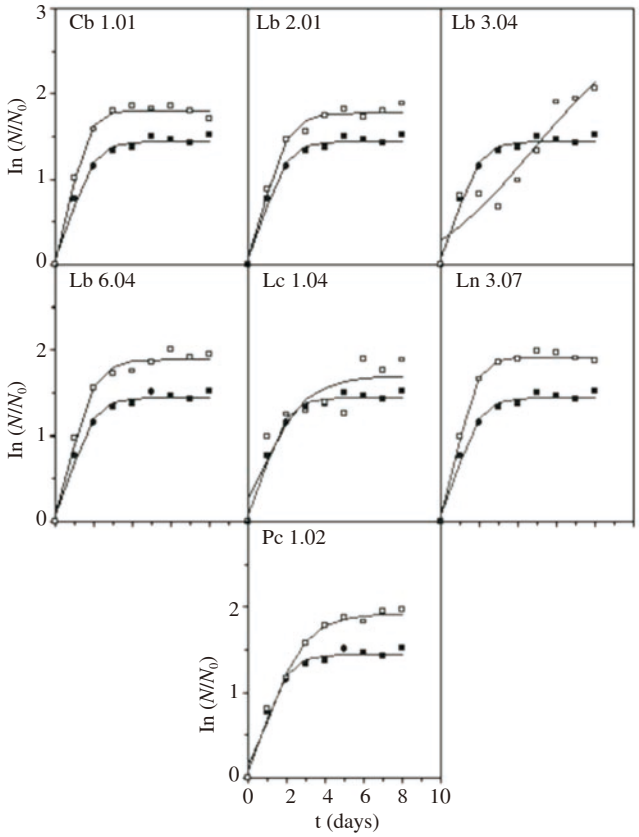

Figure 2. Effect of the addition of LAB on the growth of *I. galbana* in Erlenmeyer flasks (250 mL) (experiment 1).
White symbols: Addition; Black symbols: Control. Data fitted to equation [b]. Initial concentrations of LAB were Pc 1.02 = 0.225, Lc 1.04 = 0.200 and others = 0.500 g/L.

A detailed summary of the results obtained for the parameters including K, r_m, A, μ_m, λ and N_m in the applied fitting models was given in Table 2 (significant coefficient in all cases, with P = 0.05; n = 3). Important characteristics in the culture of microalgae were the duration of the lag phase, the growth rate and the final biomass achieved. For each treatment, the numerical values of the parametrical data (K, r_m, A, μ_m, λ and N_m) were transformed into points according to the procedure that 100 points were given to the treatment which provided the highest value of K, r_m, A, μ_m, λ and N_m and the lowest value of λ[32,33]. The other results were given as a relative percentage of the maximal values. Calculations were made for each strain and the total final score was calculated for each treatment except for Lb 3.04 and Lc 1.04 (Table 2). According to those calculations, the final scores of the cultures treated with LAB were 89.8%–100.0% whereas the score for the control was 82.9%. The highest final score was achieved by the strain Ln 3.07. The strain Pc 1.02 was the most effective in terms of final biomass and cell density (K, A and N_m = 100.0 points), the higher specific growth was obtained with the strain Cb 1.01 (r_m and μ_m = 100.0) and finally, the shortest latency period was achieved in the control (λ = 100.0).

In general, the bacterial load (MA medium) in LAB challenged flasks increased with time, being higher (about 10^7–10^8 CFU/mL) than those in control flasks (10^4–10^6 CFU/mL) throughout the experiment (Figure 3). The density for most LAB strains (MRS media) in experimental flasks remained between 10^5 and 10^8 CFU/mL for the first 4 days of culture. Lb 3.04, Lc 1.04 and Cb 1.01

Table 2
Parameters estimated for equations [a] and [b] (experiment 1).

Groups	K [a], [max (%)]	r_m [a], [max (%)]	A [b], [max (%)]	μ_m [b], [max (%)]	λ [b], [max (%)]	N_m [b], [max (%)]	Total ($\sum$ = 600)	Rank	Best (%)
Control	4.189 (61.7)	1.025 (64.4)	1.450 (75.4)	0.728 (60.1)	0.040 (100.0)	4.088 (62.0)	423.6	6	82.9
Cb 1.01	5.907 (87.0)	1.591 (100.0)	1.808 (94.0)	1.212 (100.0)	0.175 (22.9)	5.849 (88.9)	492.8	2	96.4
Lb 2.01	5.873 (86.5)	1.015 (63.8)	1.777 (92.4)	0.888 (73.3)	0.070 (57.1)	5.671 (86.0)	459.1	5	89.8
Lb 3.04	15.319 (-)	0.341 (-)	3.692 (-)	0.263 (-)	-0.326 (-)	38.465 (-)	-	-	-
Lb 6.04	6.584 (96.9)	0.983 (61.8)	1.882 (88.1)	0.974 (80.4)	0.067 (59.7)	6.294 (95.9)	482.8	3	94.5
Lc 1.04	8.082 (-)	0.324 (-)	1.695 (-)	0.485 (-)	-0.506 (-)	5.224 (-)	-	-	-
Ln 3.07	6.612 (97.3)	1.499 (94.2)	1.920 (99.8)	1.191 (98.3)	0.181 (22.1)	6.543 (99.4)	511.1	1	100.0
Pc 1.02	6.793 (100.0)	0.814 (51.2)	1.923 (100.0)	0.627 (51.7)	0.062 (64.5)	6.563 (100.0)	467.4	4	91.4

survived until the end of the experiment (10^4 and 10^6 CFU/mL, respectively at Day 8) whereas all other LAB were not detected beyond Day 4. There were neither consistent trends nor differences among the bacterial genus associated to each of the treatments. Although an important variability in bacteria strains was noticed among flasks, *Pseudomonas* and *Moraxella* were the most significant bacteria in the cultures, reaching final levels of about 10^7 CFU/mL. The former was detected through the culture period, whereas the later was identified only at Days 6–8. Interestingly, *L. mesenteroides* spp. *mesenteroides* showed an inhibitory effect on *Moraxella*.

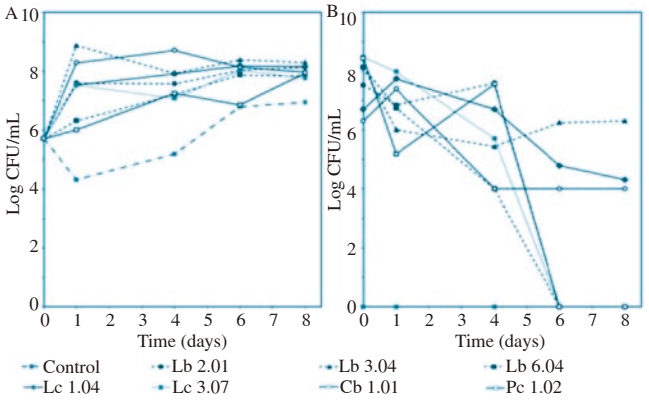

Figure 3. Effect of LAB administration on the total bacterial load (MA and MRS media) in *I. galbana* cultures (250 mL Erlenmeyer flasks, experiment 1).
A: MA media; B: MRS media; Data are expressed as log CFU/mL.

In experiment 2, two strains of LAB (Ln 3.07 and Pc 1.02) were selected from the results obtained in experiment 1 and their effects on the growth of the microalgae were studied in 5 L flasks, both with and without the addition of nutrients. Those strains provided the best growth (Ln 3.07) and the higher final biomass (Pc 1.02) in experiment 1. In experiment 2, the concentration of LAB was reduced one half for economical purposes.

After performing an analysis similar to that carried out in experiment 1 (Table 3), it can be concluded that the growth of *Isochrysis* cultures supplemented with LAB (Figure 4) resulted notoriously enhanced with respect to controls (Figure 3). The addition of Pc 1.02 and Ln 3.07 promoted a maximum final population increase (value of the parameter K in [a] or N_m in [b]) of 16% and 40%, respectively, in relation to the value of the control cultures. The strain (Ln 3.07) caused the highest parametric global improvement, reaching a final cell density of about 10^7 cells/mL.

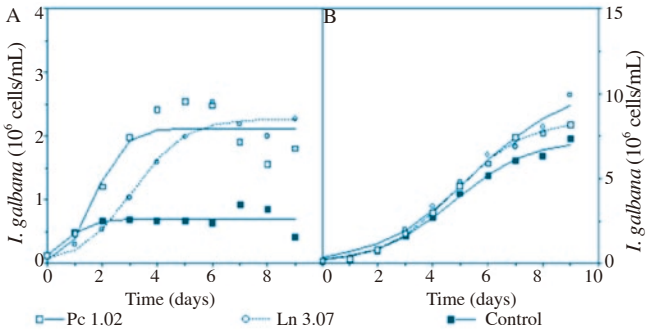

Figure 4. Effect of the addition of *L. mesenteroides* spp. *mesenteroides* (Ln 3.07) and *P. acidilactici* (Pc 1.02) on the growth of *I. galbana* in 5 L flasks in the absence (A) or presence (B) of nutrients (experiment 2).
Data fitted to equation [a]. Initial concentrations of LAB were Pc 1.02 = 0.125 and Ln 3.07 = 0.250 g/L. Different *Y*-axis scale were expressed in both figures.

In the absence of nutrients, the growth of the microalgae in LAB cultures from experiment 2 improved in a global way with respect to control cultures (total final score: 72.2%–100.0%) (Figure 3 and Table 3). The culture density in control flasks increased steadily but slowly during the first 48 h (0.7×10^6 cells/mL). Afterwards, cell density in controls remained almost constant until the end of the experiment. The final *Isochrysis* densities achieved with the addition of probiotics were 3-fold higher than in controls (2.1×10^6–2.2×10^6 cells/mL).

4. Discussion

For the quantification and evaluation of the stimulative effect, the data were submitted to a mathematical model using the logistic and the modified Gompertz models and a subsequent cumulative

Table 3
Parameters estimated for equations [a] and [b] (experiment 2).

Groups		K [a], [max (%)]	r_m [a], [max (%)]	A [b], [max (%)]	μ_m [b], [max (%)]	λ [b], [max (%)]	N_m [b], [max (%)]	Total ($\sum$ = 600)	Rank	Best (%)
Without nutrients	Control	0.696 (30.8)	2.350 (100.0)	1.692 (57.2)	2.140 (100.0)	0.264 (46.2)	0.676 (29.3)	363.5	3	72.2
	Ln 3.07	2.262 (100)	1.087 (46.3)	2.958 (100.0)	0.842 (39.3)	0.122 (100.0)	2.310 (100.0)	485.6	2	96.5
	Pc 1.02	2.122 (93.8)	1.922 (81.8)	2.867 (96.9)	1.629 (76.1)	0.189 (64.6)	2.084 (90.2)	503.4	1	100.0
With nutrients	Control	7.282 (69.9)	0.740 (94.3)	3.905 (95.4)	0.926 (97.5)	0.314 (71.7)	6.950 (79.1)	507.9	3	88.2
	Ln 3.07	10.418 (100.0)	0.600 (76.4)	4.094 (100.0)	0.943 (99.3)	0.225 (100.0)	8.786 (100.0)	575.7	1	100.0
	Pc 1.02	8.441 (81.0)	0.785 (100.0)	3.952 (96.5)	0.950 (100.0)	0.428 (52.6)	8.115 (92.4)	522.5	2	90.8

study of the parameters. Although all the strains of LAB tested in this study stimulated the growth of *I. galbana* in small volume flasks (250 mL), the best overall results and the maximal final cell densities were achieved with strains *L. mesenteroides* spp. *mesenteroides* (Ln 3.07) and *P. acidilactici* (Pc 1.02), respectively. It is interesting to point out that in a previous study[12], the growth rate of rotifers was also enhanced by the addition to the rotifers culture media of the same LAB strains tested in the present study. In rotifers, the best results were achieved with the delivery of Lc 1.04, Pc 1.02 and, particularly Lb 3.04.

With respect to strains Lb 3.04 and Lc 1.04, it is very likely that the turbidity that appeared in the small volume flasks during the first 2 days of culture could be reduced or minimized by lowering the initial bacteria concentrations. In this regard, a short-time algicidal effect at the onset of the culture should not be discarded as reported in *Gymnodinium catenatum*, *Chattonella marina* and *Heterosigma akashiwo*[34], in which a *Pseudoalteromonas* strain caused rapid cell lysis and death (within 3 h) of the algae. Over the subsequent 24 h, the algal cultures recovered.

In medium sized flasks (5 L), Ln 3.07 performed better than Pc 1.02, both with and without the addition of nutrients in the culture media. Obviously, the performance of the cultures was lower when nutrients were not added to the culture media. However, the differences in the relative performances of *I. galbana* cultures deprived of nutrients with respect to control flasks (no bacteria added) were higher than in nutrients-added flasks. This fact should be explained by unknown nutritional factors. Indeed, other authors have suggested that growth enhancement by certain bacteria starins in some species of microalgae may be caused by nutrient regeneration, control over other co-existing bacterial strains and production of stimulative substances[23,24,35-39]. In other studies, stimulative effects of bacteria on *I. galbana* cultures were not observed. Avendaño and Riquelme did not find an enhancement of *I. galbana* cultures with the addition of the probiotic bacteria *Vibrio* sp. C33, *Pseudomonas* sp. 11 and *Arthrobacter* sp. 77[5]. Furthermore, it has been also reported that cells of *I. galbana* can be lysed by strains of the genera *Saprospira* or *Pseudoalteromonas*[16,34].

Our study confirms that all the strains of LAB tested are growth-promoting bacteria for the microalgae *I. galbana*. However, the enhancement or inhibition of a given bacteria strain on diverse microalgae species seems to rely on the fact that growth factors of bacteria for microalgae are species-specific[20,34,38,40]. In this sense, Suminto and Hirayama found a bacterial strain (*Flavobacterium* DN-10) that promoted growth in semi-mass cultures of *Chaetoceros gracilis* but not in cultures of *I. galbana* and *Pavlova lutheri*[20,38]. Consequently, the results obtained in our study should not necessarily promote growth in species of microalgae other than *I. galbana*.

The use of telluric bacteria on the production of microalgae is very limited. Gonzalez and Bashan reported that the growth of *Chlorella* was improved with the use of the plant growth-promoting bacterium *Azospirillum brasilense* in small alginate beads[25]. Our results showed that the addition of terrestrial LAB strains may contribute to a fast growth of *I. galbana*, and probably other strains, with a lower production cost when the cutlure conditions and the bacteria culture media were optimised. On the other side, the artificial biomanipulation of bacterial communities in the culture of microalgae can make cultures more stable and predictable[1]. However, possible inhibitory effects of bacteria in algal cultures must also be taken into account and previous studies should be undertaken for each bacteria strain and microalgae species to discard undesirable negative effects[15,21].

Although the present study has been focussed on the growth of the microalgae *I. galbana* in culture conditions, it must be pointed out that some of the LAB strains tested (*Lactobacillus*, *Lactococcus*, *Pediococcus*, *Carnobacterium*) have been used with some success as probiotics in the control of the microflora and in the rearing of different marine organisms[4,7-12,41-43]. From our study, however, it is not possible to conclude whether LAB treatments enhanced the bacterial load in *I. galbana* cultures, due to the high variability observed in the experiments. Nevertheless, *L. mesenteroides* spp. *mesenteroides* hampered *Moraxella* growth. Similarly, Villamil *et al*. reported a decrease in the level of *Vibrio alginolyticus* in *Artemia nauplii* kept for 24 h in the presence of *L. brevis* Lb 2.01 and hence, it is likely that the addition of LAB would probably contribute to the reduction of some Vibrionaceae strains in microalgae cultures[11].

In summary, all the strains of LAB tested were found to be growth promoters for the culture of the microalgae *I. galbana*. From a practical point of view, among the strains used in this study, *P. acidilactici* (Pc 1.02) should be selected for improving final biomass, whereas maximal growth rates would be achieved with the addition of strains *C. piscicola* (Cb 1.01) and *L. mesenteroides* spp. *mesenteroides* (Ln 3.07). The best overall performance was attained in the later.

Conflict of interest statement

We declare that we have no conflict of interest.

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References

[1] Fukami K, Nishijima T, Ishida Y. Stimulative and inhibitory effects of bacteria on the growth of microalgae. *Hydrobiologia* 1997; **358**: 185-91.

[2] Naviner M, Bergé JP, Durand P, Le Bris H. Antibacterial activity of the marine diatom *Skeletonema costatum* against aquacultural pathogens. *Aquaculture* 1999; **174**: 15-24.

[3] D'Alvise PW, Lillebø S, Prol-Garcia MJ, Wergeland HI, Nielsen KF, Bergh Ø, et al. *Phaeobacter gallaeciensis* reduces *Vibrio anguillarum* in cultures of microalgae and rotifers, and prevents vibriosis in cod larvae. *PLoS One* 2012; **7**(8): e43996.

[4] Ringø E, Gatesoupe FJ. Lactic acid bacteria in fish: a review. *Aquaculture* 1998; **160**: 177-203.

[5] Avendaño RE, Riquelme CE. Establishment of mixed-culture probiotics

and microalgae as food for bivalve larvae. *Aquac Res* 1999; **30**: 893-900.

[6] Gatesoupe FJ. The use of probiotics in aquaculture. *Aquaculture* 1999; **180**: 147-65.

[7] Gomez-Gil B, Roque A, Turnbull JF. The use and selection of probiotic bacteria for use in the culture of larval aquatic organisms. *Aquaculture* 2000; **191**: 259-70.

[8] Verschuere L, Rombaut G, Sorgeloos P, Verstraete W. Probiotic bacteria as biological control agents in aquaculture. *Microbiol Mol Biol Rev* 2011; **64**: 655-71.

[9] Pintado J, Prol MJ, Balcazar JL, Planas M, Makridis P. New strategies for the control of bacterial infections in marine fish larval rearing. In: Montet D, Ray RC, editors. *Aquaculture microbiology and biotechnology*. Vol. 2. Florida: CRC Press; 2011, p. 1-30.

[10] Pintado J, Planas M, Makridis P. Live feeds: microbial assemblages, probiotics and prebiotics. In: Merrifield D, Ringø E, editors. *Aquaculture nutrition: gut health, probiotics and prebiotics*. Hoboken: Wiley-Blackwell; 2014, p. 419-42.

[11] Villamil L, Figueras A, Planas M, Novoa B. Control of *Vibrio alginolyticus* in *Artemia* culture by treatment with bacterial probiotics. *Aquaculture* 2003; **219**: 43-56.

[12] Planas M, Vázquez JA, Marqués J, Pérez-Lomba R, González MP, Murado M. Enhancement of rotifer (*Brachionus plicatilis*) growth by using terrestrial lactic acid bacteria. *Aquaculture* 2004; **240**: 313-29.

[13] Zink IC, Douillet PA, Benetti DD. Improvement of rotifer *Brachionus plicatilis* population growth dynamics with inclusion of *Bacillus* spp. probiotics. *Aquac Res* 2013; **44**: 200-11.

[14] Berland BR, Bonin DJ, Maestrini SY. Study of bacteria associated with marine algae in culture. III. organic substrates supporting growth. *Mar Biol* 1970; **5**: 68-76.

[15] Baker KH, Herson DS. Interactions between the diatom *Thallasiosira pseudonanna* and an associated pseudomonad in a mariculture system. *Appl Environ Microbiol* 1978; **35**: 791-6.

[16] Sakata T. Occurrence of marine *Saprospira* sp. possessing algicidal activity for diatoms. *Nippon Suisan Gakkaishi* 1990; **56**: 1165.

[17] Sakata T, Fujita Y, Yasumoto H. Plaque formation by algicidal *Saprospira* sp. on a lawn of *Chaetoceros ceratosporum*. *Nippon Suisan Gakkaishi* 1991; **57**: 1147-52.

[18] Imai I, Ishida Y, Sawayama S, Hata Y. Isolation of a marine gliding bacterium that kills *Chattonella antique* (Raphidophyceae). *Nippon Suisan Gakkaishi* 1991; **57**: 1409.

[19] Mitsutani A, Takesue K, Kirita M, Ishida Y. Lysis of *Skeletonema costatum* by *Cytophaga* sp. isolated from the coastal water of the Ariake Sea. *Nippon Suisan Gakkaishi* 1992; **58**: 2159-67.

[20] Suminto, Hirayama K. Relation between diatom growth and bacterial population in semi mass culture tanks of diatom. *Bull Fac Fish Nagasaki Univ* 1993; **74/75**: 37-41.

[21] Suminto, Hirayama K. Effects of bacterial coexistence on the growth of a marine diatom *Chaetoceros gracilis*. *Fish Sci* 1996; **62**: 40-3.

[22] Fukami K, Yuzawa A, Nishijima T, Hata Y. Isolation and properties of a bacterium inhibiting the growth of *Gymnodinium nagasakiense*. *Nippon Suisan Gakkaishi* 1992; **58**: 1073-7.

[23] Riquelme CE. Interaction between microalgae and bacteria in coastal seawater [dissertation]. Kyoto: Kyoto University; 1988, p. 1-92.

[24] Riquelm CE, Fukami K, Ishida Y. Effects of bacteria on the growth of a marine diatom, *Asterionella glacialis*. *Bull Jpn Soc Microb Ecol* 1988; **3**: 29-34.

[25] Gonzalez LE, Bashan Y. Increased growth of the microalga *Chlorella vulgaris* when coimmobilized and cocultured in alginate beads with the plant-growth-promoting bacterium *Azospirillum brasilense*. *Appl Environ Microbiol* 2000; **66**: 1527-31.

[26] Cabo ML, Murado MA, González MP, Pastoriza L. A method for bacteriocin quantification. *J Appl Microbiol* 1999; **87**: 907-14.

[27] Walne PR. *Experiments in the large-scale culture of the larvae of Ostrea edulis L.* London: Her Majesty's Stationery Office; 1966, p. 53.

[28] Vázquez JA. Lactobacterias como probiontes e produtoras de bacteriocinas. Modelos de crecemento e actividade. Aplicacións á acuicultura. Ph.D. Thesis University of Santiago of Compostela, Spain; 2001.

[29] Blanch HW, Clark DS. Biochemical engineering. New York: Marcel Dekker Inc; 1997, p. 185.

[30] Zwietering MH, Jongenburger I, Rombouts FM, van 't Riet K. Modelling of the bacterial growth curve. *Appl Environ Microbiol* 1990; **56**: 1875-81.

[31] Buck JD. Nonstaining (KOH) method for determination of Gram reactions of marine bacteria. *Appl Environ Microbiol* 1982; **44**: 992-3.

[32] Dufossé L, De La Broise D, Guerard F. Evaluation of nitrogenous substrates such as peptones from fish:a new method based on Gompertz modeling of microbial growth. *Curr Microbiol* 2001; **42**: 32-8.

[33] Vázquez JA, González MP, Murado MA. A new marine medium: use of different fish peptones and comparative study of the growth of selected species of marine bacteria. *Enzyme Microb Technol* 2004; **35**: 385-92.

[34] Lovejoy C, Bowman JP, Hallegraeff GM. Algicidal effects of a novel marine *Pseudoalteromonas* isolate (class Proteobacteria, gamma subdivision) on harmful algal bloom species of the genera *Chattonella*, *Gymnodinium*, and *Heterosigma*. *Appl Environ Microbiol* 1998; **64**: 2806-13.

[35] Haines KC, Guillard RRL. Growth of vitamin B_{12}-requiring marine diatoms in mixed laboratory cultures with vitamin B_{12}-producing marine bacteria. *J Phycol* 1974; **10**: 245-52.

[36] Ukeles R, Bishop J. Enhancement of phytoplankton growth by marine bacteria. *J Phycol* 1975; **11**: 142-9.

[37] Cole JJ. Interaction between bacteria and algae in aquatic ecosystem. *Annu Rev Ecol Syst* 1982; **13**: 291-314.

[38] Suminto, Hirayama K. Application of a growth-promoting bacteria for stable mass culture of three marine microalgae. *Hydrobiologia* 1997; **358**: 223-30.

[39] Rico-Mora R, Voltolina D, Villaescusa-Celaya JA. Biological control of *Vibrio alginolyticus* in *Skeletonema costatum* (Bacillariophyceae) cultures. *Aquac Eng* 1998; **19**: 1-6.

[40] Fukami K, Nishijima T, Murata H, Doi S, Hata Y. Distribution of bacteria influencial on the development and the decay of *Gymnodinium nagasakiense* red tide and their effects on algal growth. *Nippon Suisan Gakkaishi* 1991; **57**: 2321-6.

[41] Gatesoupe FJ. The effect of three strains of lactic bacteria on the production rate of rotifers, *Brachionus plicatilis*, and their dietary value for larval turbot, *Scophthalmus maximus*. *Aquaculture* 1991; **96**: 335-42.

[42] Gatesoupe FJ. Probiotic and formaldehyde treatments of *Artemia nauplii* as food for larval pollack, *Pollachius pollachius*. *Aquaculture* 2002; **212**: 347-60.

[43] Hansen GH, Olafsen JA. Bacterial interactions in early stages of marine cold water fish. *Microb Ecol* 1999; **38**: 1-26.