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Ascophillum nodosum and *Lithothamnium calcareum* and their prebiotic potential on *Lactobacillus strains*

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A R T I C L E I N F O

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A B S T R A C T

Functional ingredients became essential for sustainable development to improve health status, prevent disease, and reduce the use of medication. This study aimed to determine the potential prebiotic role of *Ascophyllum nodosum and Lithothamnium calcareum* using *Lactiplantibacillus plantarum* and *Limosilactobacillus reuteri* as microorganism models. Key bioactive compounds of algae were identified using LC-QTOF-MS/MS. The influence of algae inclusion on the growth of *Lactobacilli strains* was evaluated. Functional activities of the co-culture were evaluated after 24, 48, 72, and 96 incubation hours. Antioxidant capacity (by ABTS assay) increased for both *Lactobacilli* cultures, with a PI% increase of 30 % for *L. plantarum* ($p < 0.01$) and a PI% increase of 25 % for *L. reuteri* ($p < 0.01$). Algal extracts significantly inhibited ($p < 0.05$) *E. coli* growth after 48 hrs for *L. plantarum* and after 72 hrs for *L. reuteri*. The results suggested that *A. nodosum* and *L. calcareum* can be considered valid prebiotics for both strains.

1. Introduction

Sustainable development has been broadly discussed nowadays due to the degradation of natural resources and environmental changes mainly caused by the agro-industrial sector. Moreover, the rapid world population growth in the upcoming years (9.8 billion in 2050) (FAO, 2019) increases food demand, with 70 % for animal products (Alexandratos and Bruinsma, 2012). Thus, the concept of sustainability requires to be applied to animal feed, which is the primary input of food production of animal origin. Therefore, solutions that intensify food production, with a simultaneous reduction in production costs and greater sustainability according to Agenda 2030 goals, are needed (UN General Assembly, 2015). The reduction of antibiotics plays a pivotal role in this scenario, and the concept of functional additives and ingredients has gained increasing importance in both humans and animals (Adefegha, 2018; Markowiak & Śliżewska, 2018; Terpou et al., 2019). The growing importance of functional ingredients in various industries, particularly in areas such as animal nutrition and human nutrition can provide both nutritional and beneficial effects of this kind of ingredients, due to their anti-inflammatory, antioxidant, antimicrobial, and antiviral properties. Therefore, these substances may reduce the overuse of antibiotics (European Commission, 2019). According to the European Food Safety Authority (EFSA, 2012), probiotics are important alternatives among the various substances. Growth of probiotics can be implemented following the intake of ingredients with prebiotic potential. However, it makes them poorly digestible or fermentable in the host intestinal microflora. The prebiotic approach, therefore, aims to increase beneficial intestinal bacteria such as *Lactobacillus* and *Bifidobacterium* (Parracho et al., 2007). Recent literature results demonstrated the beneficial effects induced by the intake of macroalgae and microalgae due to their wide range of bioactive compounds, such as proteins, polysaccharides, pigments, vitamins, and polyunsaturated fatty acids (PUFAs) (Gouveia et al., 2008; Raposo et al., 2013; Vidanarachchi et al., 2012). Among these, polysaccharides (PS) and their derivatives (such as soluble fibers) are the compounds that have revealed the most significant prebiotic potential. Some of these PS (for example, polysaccharides, fucoidan, alginates, and carrageenans) are not entirely digested by intestinal metabolic enzymes and, therefore, can act as prebiotics (Zaporozhets et al., 2014). Within certain algal biomasses, specific PS with prebiotic potential has been identified to play an essential role in the regulation of

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intestinal health (Patel et al., 2021). Brown macroalgae (class Phaeophyceae) are rich in non-digestible polysaccharides such as alginates, laminarins, and fucoidan, account for more than 50 % of the total dry weight of brown algae, and can reach up to 70 % in some species (de Jesus Raposo et al., 2015; Holdt & Kraan, 2011). Red algae (class Florideophycea) are also rich in polysaccharides, and their content varies between 10 % and 50 % depending on the species and the part of the plant (Makkar and Ankers, 2014).

Although the polysaccharide class has been identified as the major contributor to the prebiotic capacity of certain algal species, little is known about how the co-presence of algae and lactobacilli may influence the different bioactive components and, consequently, functional properties such as antioxidant and antimicrobial activity. This aspect plays a key role in the field of nutrition, where the current aim is to introduce on the animal and human market products that can protectively enhance the health, thereby minimizing the need of excessive use of medicines and antibiotics. This approach aims to mitigate the concern of antibiotic resistance phenomen.

Additionally, there is also a clear need for a more holistic approach that highlights the theoretical basis by considering more the potential synergistic effects achievable when different functional ingredients are combined.

Therefore, this study aims to evaluate the prebiotic potential of two algal species, *Ascophyllum nodosum* and *Lithothamnium calcareum in vitro* (Frazzini et al., 2022), against two probiotic strains generally present in the intestinal flora, *Lactobacillus plantarum* and *Lactobacillus reuteri*. Moreover, the study aims to determine the total polyphenol content, total flavonoid content, and TOF-LC/MS-MS characterization of bioactive compounds from those algae species.

2. Materials and methods

2.1. Materials

Ascophyllum nodosum (AN; product code: 01.2000051) and *Lithothamnium calcareum* (LC, product code: 01.2000143) were purchased from Italfeed Srl (Milan, Italy) in confirmation with European safety requirements. All algal species and their combination (50:50; w/w) were 100 % pure powder. Before further experiments, the extraction method based on ethanol were proceeded.

2.2. Extraction of algal biomass procedure

Extracts were prepared according to Zhong B. et al. (Zhong et al., 2020). Briefly, 2 g of each seaweed was rubbed and mixed with 10 mL of ethanol (80:20 v/v). Then, incubation was carried out at 4 °C for 16 h in agitation. After that, all the samples were centrifuged at 10,000 rpm for 10 min. The supernatant was filtered through a 0.20 μm syringe RC filter, collected, and stored at −20 °C for further analysis.

2.3. TOF-LC/MS-MS characterization of bioactive compounds

HPLC and mass spectrometric analyses (LC–MS2) were analyzed according to Zhong B. et al. (Zhong et al., 2020) with minor modifications. Briefly, samples were analyzed through an HPLC (Sciex, Massachusetts, USA) equipped autosampler (Sciex, Massachusetts, USA) coupled to a quadrupole time-of-flight mass spectrometer (Q-TOF-MS) (X500R QTOF, Sciex, Massachusetts, USA). The separation was achieved by a Synergy Hydro-RP 80 Å, LC Column (250 mm 4.6 mm, 4 m) (Phenomenex, Lane Cove, NSW, Australia). 10 μL of the sample was injected by an autosampler and eluted through the column with a binary mobile phase consisting of A (water containing 0.1 % formic acid) and B (acetonitrile containing 0.1 % formic acid). The flow rate of 0.4 mL/min was used. A 35 min linear gradient was programmed as follows: 0–5 min, 0.5 % A, 5 % B; 5–25 min, 75 % A, 25 % B; 25–31 min, 5 % A, 95 % B; 31–35 min, 95 % A, 5 % B. Compounds were identified by comparing retention time (RT) and UV spectra information, and confirmed by HPLC-Q-TOF-MS/MS. Data were analyzed through SCIEX OS version 3.1 for peak comparison and molecular compound identification in two different libraries: i) metabolites ii) natural compounds (Sciex, Massachusetts, USA).

2.4. Bacterial strains and culturing conditions

L. plantarum and *L. reuteri* strains were individually inoculated from our laboratory stock at − 80 °C (Dell'Anno et al., 2021) into the De Man, Rogosa and Sharpe (MRS) medium (Liofilchem, Italy) and incubated at 35 °C for 24 h under a microaerophilic atmosphere.

2.5. Growth of Lactiplantibacillus plantarum and Limosilactobacillus reuteri cultured in the presence of Ascophyllum nodosum and Lithothamnium calcareum

The effect of algae, alone and in combination (AN; LC; AN-LC), on the growth of *Lactiplantibacillus plantarum* and *Limosilactobacillus reuteri* was assessed by incubating the 24-h *Lactobacillus* strains with a density of 10^7 CFU/mL, in MRS broth (Merck, Germany), in the presence of algae at concentrations of 0.5 % (w/v), 1.0 % (w/v) and 1.5 % (w/v) (Scieszka et al., 2020). The growth of *L. plantarum* and *L. reuteri* was determined via measurement of the optical density of each culture at 600 nm (OD600) at specific intervals (0, 4, 6, 8, 10, and 12 mins) during incubation at 35 °C in a spectrophotometer (P-800 Scan Ready, Life Real, All obtained data were converted into $\log_{10}$ of the number of CFU/mL, by a calibration curve (considering 1 OD equal to 10^9 CFU/mL) (Trabelsi et al., 2013). The assay was performed in two biological replicates and technical triplicate replicates.

2.6. Co-culture of Lactiplantibacillus plantarum and Limosilactobacillus reuteri and algae extract

Lactiplantibacillus plantarum and *Limosilactobacillus reuteri* strain belonging to our strain library (Dell'Anno et al., 2021) was grown at 37 °C overnight in MRS broth (Merck, Germany). After the cultivation, the bacterial cells were collected by centrifugation and resuspended with sterile 0.85 % (w/v) NaCl solution. AN and LC were extracted as described above. Then, the *L. plantarum* and *L. reuteri* cells suspended in the sterile 0.8 % (w/v) NaCl solution were inoculated at a final 1.0 % v/v in the algae extract and incubated for different time points (24, 48, 72, and 96 hrs) at 37 °C. Past the established incubation time, the co-culture was centrifuged, filtered with a 0.20 μm syringe filter, and stored at −20 °C for further analysis (Shakya et al., 2021).

2.7. Evaluation of Total polyphenol content (TPC)

The phenolic content of extracts resulting from co-culture was evaluated by the Folin-Ciocalteu method, according to Shakya (Shakya et al., 2021). Briefly, 10 μL of extract plus 100 μL of Folin-Ciocalteu reagent (diluted 1:10 with distilled water) were added to a 96-well plate. After 3 min, 90 μL sodium carbonate (1 M) was added, and the plate stood for 1 h. Total phenolic content was determined spectrophotometrically at 765 nm (JASCO V-630 UV–vis, Germany). Each sample and standard were run in triplicate. The Total Phenolic Content (TPC) was expressed as Tannic Acid Equivalent (mg TAE/100 g).

2.8. Evaluation of Total flavonoid content (TFC)

The flavonoid content of extracts resulting from co-culture was measured according to Herald, T.J. (Herald et al., 2012). Briefly, 250 μL of extract, 1 mL of distilled water, and 75 μL of $NaNO_2$ (50 g/L) were combined and mixed. Subsequently, 150 μL of $AlCl_3$ (100 g/L) was added to the solution. After 6 min of incubation, 500 μL of NaOH (1 mol/L) and 500 μL of distilled water were added. The solution was

centrifuged at 3220 g for 5 min at RT. Absorbance was measured at 510 nm. Each sample and standard were run in triplicate. Total flavonoid concentration was expressed as mg catechin equivalent (CE) g^{-1} sample.

2.9. Evaluation of antioxidant activity through ABTS radical scavenging activity

ABTS assay was employed to determine the radical scavenging activity of the extracts, similar with our previous studies (Frazzini et al., 2023). Briefly, 10 μL of extract was added to 1 mL of $ABTS^{+}$ working solution. After 6 min in the dark, absorbance was measured spectrophotometrically at 734 nm. All assays were performed in technical triplicate and with two biological replicates to verify the replicability. The radical scavenging activity was expressed as the percentage of the inhibition of radical scavenging activity (PI%) according to the following equation:

$$PI(\%) = \left(\frac{AbsBlank - AbsSample}{AbsBlank}\right) * 100$$

2.10. Evaluation of antimicrobial activity

The antimicrobial activity was evaluated according to previous research in our group (Frazzini et al., 2023). In particular, we performed the growth inhibition assay against *Escherichia coli* $F18^{+}$. Briefly, 100 μl of extracts derived from co-cultures was added to a 96-well plate with 30 μL of *E. coli* inoculum. The negative controls were used to correct the background color. All samples were then incubated at 37 °C in a shaking incubator for 6 h. The absorbance was recorded every 60 min with a microplate reader spectrophotometer (ScanReady P-800, Life Real, Hangzhou, China) at an optical density (OD) of 620 nm. The measured OD was converted into $\log_{10}$ of the number of cells/mL, considering 1 OD equals 10^9 cells/mL (Myers et al., 2013). All assays were performed in a technical quadruplicate and with three biological replicates. Afterward, Minimal Inhibitory concentration (MIC) was evaluated. Briefly, 100 μL of samples were plated in a 96-well microplate, and 10 μL of an overnight culture of *E. coli* $F18^{+}$ (approximately 10^6 CFU/mL) was added in each well, except for the blank and negative control ($CTRL^{-}$) wells, and incubated at 37 °C for 20 h. The change in absorbance determined bacterial growth after reading the microplates at 620 nm in a microplate reader spectrophotometer (Scan-Ready P-800, Life Real, Hangzhou, China). The following formula estimated the inhibition rate:

$$Inhibitionrate(\%) = 100 * \left(\frac{ODCTRL^{-} - ODsample}{ODCTRL^{-} - ODblank}\right)$$

2.11. Statistical analysis

All the data were analyzed using GraphPad Prism software (Version 9.0.0). Statistical analysis was performed after evaluating the normal distribution of data through Shapiro-Wilk and D'Agostino-Pearson tests. Concerning data obtained from functional activity assessment assays, a two-way analysis of variance (ANOVA) was performed, evaluating the effect of treatment, time, and their interaction. Post hoc pairwise comparisons were performed using the Bonferroni-Sidak test. Data were reported as mean ± standard error, and differences were considered statistically significant for $p < 0.05$.

3. Results and discussion

3.1. TOF-LC/MS-MS characterization of bioactive compounds

Metabolomic profile analysis carried out by HPLC-QTOF-MS/MS revealed the presence of different molecules with bioactive properties in the extract of the combination of *Ascophyllum nodosum* and *Lithothamnium calcareum* (Table 1). The results indicated that the combination of the two algae species contains several important bioactive molecules. Among them, we have found hydroxybenzoic acid, which has antioxidant activity against free radicals (Heleno et al., 2015), antimicrobial activity against pathogenic bacteria and fungi (Heleno et al., 2013; Pugazhendhi et al., 2005). Another bioactive compound present in these algae was malic acid, which together with its isotopes (L-malic acid) and derivatives (2-isopropylmalic acid), are known for their bioactive properties. Malic acid exerts antioxidant power, capable of neutralizing free radicals. This molecule is able to donate an electron to free radicals, reducing them and making them stable, or alternatively can form bonds with free radicals, inhibiting their harmful effect (Lobo et al., 2010). Malic acid is also active against a wide range of bacteria, including *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, and *Pseudomonas aeruginosa*, and against some fungi, including *Candida albicans* and *Aspergillus fumigatus* (Rybczyńska-Tkaczyk et al., 2023). Malic acid demonstrates antimicrobial properties by (i) damaging the cell walls of bacteria and fungi, thus making them vulnerable to pathogens, and (ii) interfering with the metabolic processes of bacteria and fungi, preventing them from growing and reproducing (Marques et al., 2020). Another bioactive compound is citric acid, which has antioxidant effects, primarily through its metal-chelating properties (Moon et al., 2020). Citric acid can chelate or bind metal ions such as iron and copper and can help prevent these metal ions from catalyzing oxidation reactions that produce harmful free radicals. In this way, citric acid can indirectly contribute to antioxidative effects by sequestering pro-oxidant metal ions (Van Den Berg et al., 2003). Even if citric acid does not have high antimicrobial capabilities, however its low pH benefits an acidic environment hostile to the growth of pathogenic microorganisms (Burel et al., 2021).

3.2. Growth of Lactiplantibacillus plantarum and Limosilactobacillus reuteri cultured in the presence of Ascophyllum nodosum and Lithothamnium calcareum

To consider algae as potential prebiotics against probiotics such as *Lactiplantibacillus plantarum* and *Limosilactobacillus reuteri*, the influence of algae addition on the growth curve of the lactobacilli was evaluated. Based on previous literatures studies, three different inclusion levels (0.5 %; 1.0 %; 1.5 %) were selected. These inclusions previously demonstrated no toxic effects on different strains of lactobacilli (P.-T. Chen et al., 2020; Khemiri et al., 2023; Ścieszka & Klewicka, 2020). Algae addition alone (AN and LC) and in combination (AN-LC), resulted in increased growth of *L. plantarum*. The inclusion of 0.5 % (Fig. 1c) was the most effective, with a significant increase ($p < 0.001$) from the

Table 1
Main molecules with bioactive properties present in the extract of algae (*Ascophyllum nodosum* and *Lithothamnium calcareum*) combination found by HPLC-QTOF-MS/MS analysis.

Compound identified	Formula	Area	RT (min)	$[M-H]^{-}$ (*m/z*, Da)	*m/z* (Da) of main fragments (relative intensity, %), MS/MS
2-isopropylmalic acid	C7H12O5	449,224	10.15	175.0611	85.06 (50); 113.06 (30); 115.03 (60)
4-Hydroxybenzoic acid	C7H6O3	68,174	9.16	137.0244	65.03 (20); 93.03 (100); 137.02 (10)
Citric acid	C6H8O7	7,747,811	1.80	191.0195	87.00 (80); 111.00 (100)
D-sorbitol	C6H14O6	9,104,845	1.40	181.0716	59.01 (100); 71.01 (100); 89.02 (30); 101.02 (25)
L-Malic acid	C4H6O5	34,894	1.69	133.01	71.01 (100); 72.99 (30)
Succinic acid	C4H6O4	634,340	2.06	117.0193	73.02 (100)

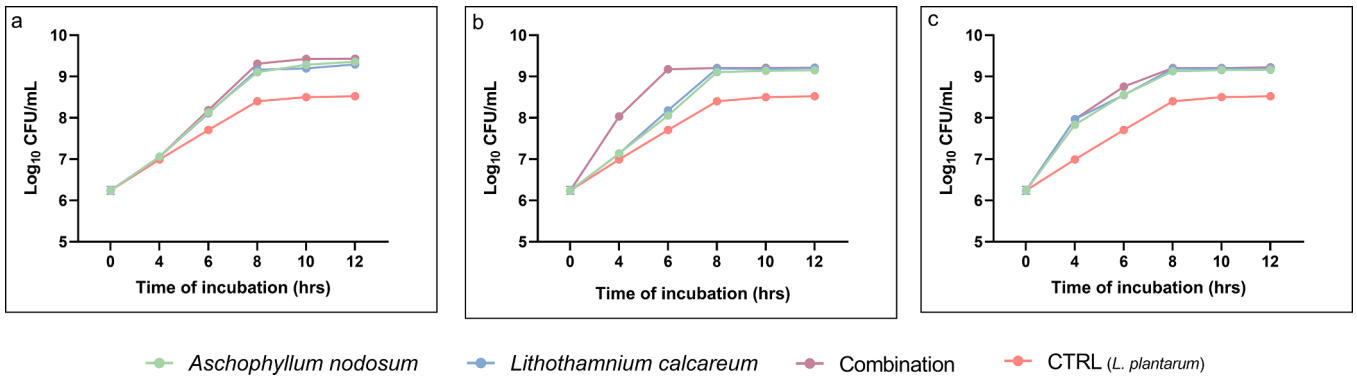

Fig. 1. Growth curve of *Lactiplantibacillus plantarum* cultured with algae species (*Ascophyllum nodosum*, *Lithothamnium calcareum* and their combination) of 1.5% of algae inclusion level (a); 1.0% of algae inclusion level (b); 0.5% of algae inclusion level (c).

fourth hour of incubation, where it reached a value of 9 $\log_{10}$ cells/mL. Increasing the inclusion level to 1.0 % and 1.5 % (Fig. 1b and Fig. 1a, respectively), the growth level remained higher than the control, represented by *L. plantarum* culture and reached the value of 9 $\log_{10}$ cells/mL only after 6 and 8 hrs of incubation, respectively. Furthermore, regarding the effect of the addition of the algal species on the growth of *Limosilactobacillus reuteri*, the results showed that high inclusion levels (Fig. 2a) did not present a linear increase of the *Lactobacillus*. The growth curve of the control (culture of *L. reuteri*) reached higher growth levels during the first few hours of incubation. As the inclusion level decreases (Fig. 2b and Fig. 2c), the growth curve becomes more linear, with an initial exponential phase, and then reaches a stationary phase after 24 h of incubation. Furthermore, the 0.5 % inclusion level did not only allow a linear growth of *L. reuteri*, but AN-LC, demonstrated that the growth curve was significantly higher ($p < 0.001$) than that of the control, thus highlighting that the co-presence of the two algal species allowed for better growth of *L. reuteri* (Fig. 2c). The obtained results align with the literature, where some studies have revealed an increase in the growth of the lactobacilli in co-culture with different algal species (P.-T. Chen et al., 2020; Khemiri et al., 2023). The increase in the growth of lactobacilli once placed in co-culture with AN and LC is due to their nature. Both *L. plantarum* and *L. reuteri* are probiotics that are naturally present in the gastrointestinal tract and bring various benefits to the host's health if present in appropriate quantity (Iorizzo et al., 2021; Z. Yu et al., 2023). Furthermore, these probiotics are two Gram-positive lactic bacteria that can produce fermented foods (Giraffa et al., 2010; Z. Wang et al., 2022). Moreover, algae are photosynthetic organisms that deliver oxygen and absorb carbon dioxide in their life cycle (Chapman, 2013; Raven & Giordano, 2014). Therefore, the growth of *L. plantarum* or *L. reuteri* can be accelerated when cultivated with algae, because algae produce oxygen, an essential nutrient for lactobacilli. In addition, algae can also absorb carbon dioxide, a waste product of lactobacilli, which is harmful to the growth environment and can also accelerate the growth of lactobacilli themselves (Cantú-Bernal et al., 2020; Gupta & Abu-Ghannam, 2011).

3.3. Evaluation of Total polyphenol content (TPC)

The evaluation of the polyphenol content after the co-culture of lactobacilli with AN and LC revealed that concerning *Lactobacillus plantarum*, the co-culture with the algal species did not significantly affect the polyphenol content compared to the algae extract (Fig. 3a). However, by comparing the different co-cultures at the same time point, the presence of AN and the combination of the two algal species led to a significant increase ($p < 0.001$) compared to the culture of the single *lactobacillus* (558.06 ± 22.76 mg TAE/g of sample). Considering the co-culture carried out between the two algal species and *L. reuteri* (Fig. 3b), the presence of the *lactobacillus* decreased ($p < 0.05$) in the polyphenol content found after the analysis of the co-cultures containing AN and the combination of the algal species compared to the values obtained from the analysis of the extract. In contrast, the polyphenol content in the co-cultures containing LC increased if compared to the corresponding extract (5.16 ± 0.49 mg TAE/g of sample and 1.94 ± 0.37 mg TAE/g of sample, respectively). Despite this decrease, the results obtained have shown that, as occurs with the co-culture with *L. plantarum*, even in this case, the polyphenol content in the co-cultures containing AN and the combination of the algal species was higher than that observed in the culture of *L. reuteri* single. Although no studies in the literature are present on the co-cultures of the algal species *L. plantarum* and *L. reuteri*, the obtained results reflected those highlighted by previous studies that have considered other algal species and other strains of lactobacilli. In fact, in a study conducted by Jamnik P. et al. (Jamnik et al., 2022), the

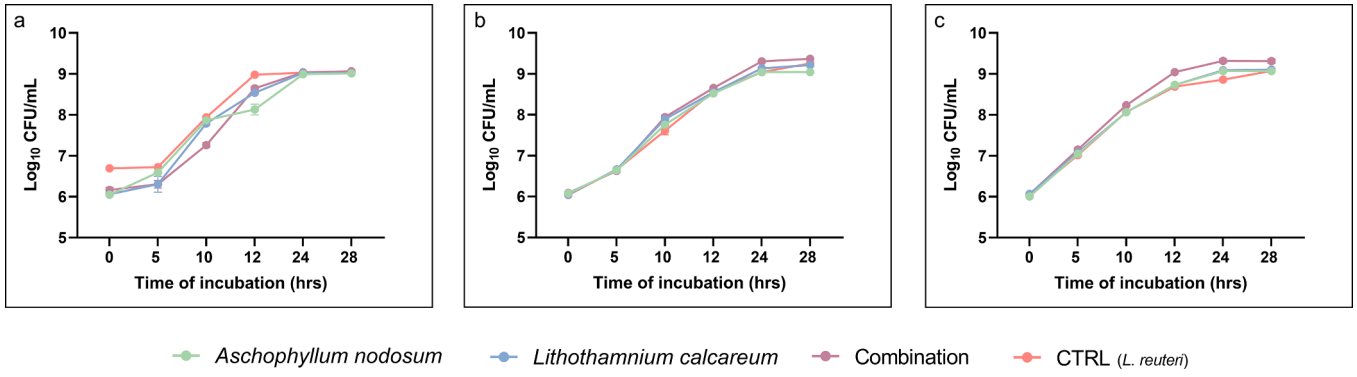

Fig. 2. Growth curve of *Limosilactobacillus reuteri* cultured with algae species (*Ascophyllum nodosum*, *Lithothamnium calcareum* and their combination) of 1.5% of algae inclusion level (a); 1.0% of algae inclusion level (b); 0.5% of algae inclusion level (c).

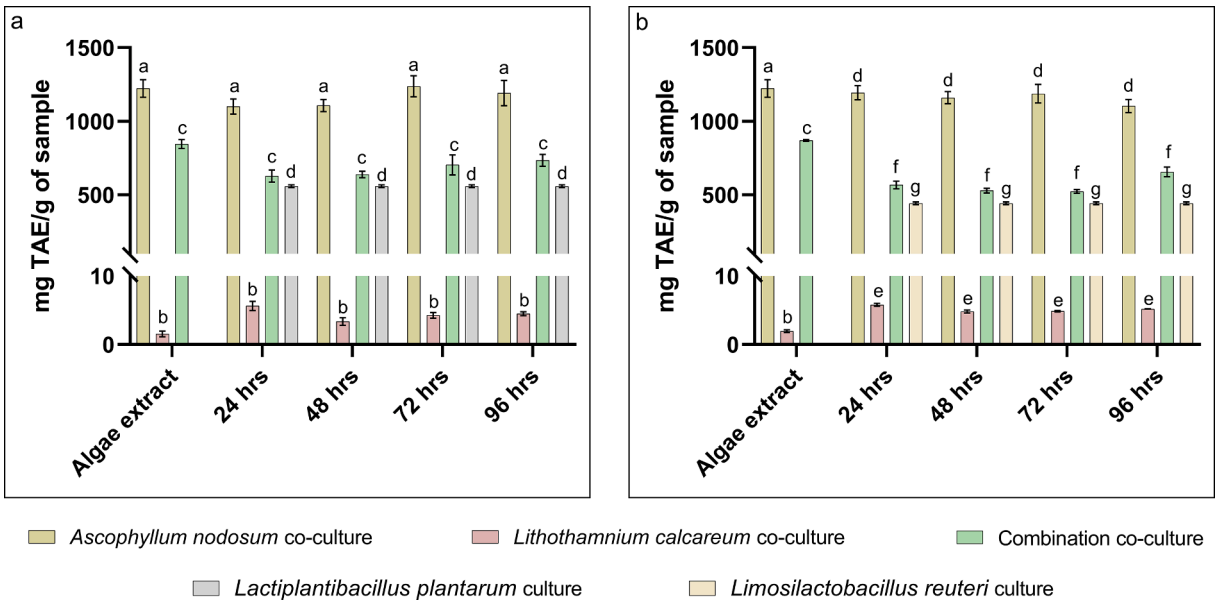

Fig. 3. Total Phenolic Content (TPC) of (a) TPC of *Lactiplantibacillus plantarum* co-culture with algal extracts; (b) TPC of *Limosilactobacillus reuteri* co-culture with algal extracts. Abbreviation: TAE = tannic acid equivalent. Data are shown as means ± standard deviations (SD) (n = 3). [a, b, c, d, e, f, g] Means with different superscript letters indicate statistically significant differences ($p < 0.05$).

co-culture of *Lactobacillus plantarum* and *Arthrospira platensis* produced a significant increase in the polyphenol content compared to the mono-bacterial culture of *L. plantarum*. A further study conducted by Li Z. et al. (Li et al., 2023) highlighted that *Bangia fusco-purpurea*, a red alga, inoculated with *Lactobacillus delbrueckii* and *Lactobacillus plantarum* showed a significant increase in the polyphenol content. In contrast to what was found for the co-cultures containing AN and the algal combination, the co-culture involving the alga LC both in the presence of *L. plantarum* and *L. reuteri*, showed a polyphenol content lower than that of the culture of the single lactobacilli (558.06 ± 22.76 mg TAE/g of sample, and 442.75 ± 21.23) (Fig. 3), due to the low polyphenol content of LC (Almeida et al., 2012). The mechanisms of actions underlying the increase in the polyphenol content in co-cultures of lactobacilli and algae need to be fully understood. However, the microorganisms may interact with each other to promote the production of polyphenols. For example, *Lactobacilli* can produce enzymes that degrade the cellulose of algae, releasing the polyphenols contained in the cell walls (Adebo & Gabriela Medina-Meza, 2020; Hadj Saadoun et al., 2021; Ricci et al., 2019; Y. Wang et al., 2019).

3.4. Evaluation of Total flavonoid content (TFC)

The flavonoid content analysis following co-culture with algae and lactobacilli revealed a general decrease in this molecular class (Fig. 4). In fact, the co-culture involving *L. plantarum* showed that after the first 48 h of incubation, the flavonoid content of the cultures containing AN

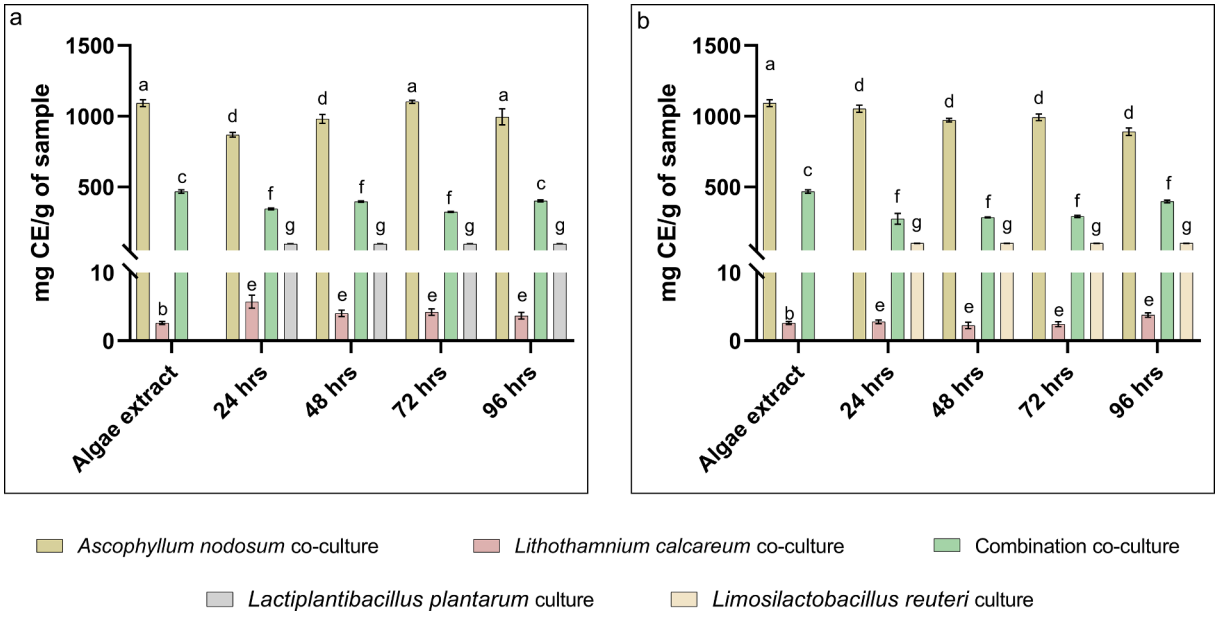

Fig. 4. Total Flavonoid Content (TFC) of TFC of *Lactiplantibacillus plantarum* co-culture with algal extracts (a); TFC of *Limosilactobacillus reuteri* co-culture with algal extracts (b). Abbreviation: CE = Catechin equivalent. Data are shown as means ± standard deviations (SD) (n = 3). [a, b, c, d, e, f, g] Means with different superscript letters indicate statistically significant differences ($p < 0.05$).

and the algal combination had significantly decreased compared to the content of the algal extracts in their pure form. The AN extract reported flavonoid values of 1091.98 ± 70.43 mg CE/g of sample, which decreased to 867.60 ± 45.97 mg CE/g of sample and 980.82 ± 88.96 mg CE/g of sample, respectively after 24 and 48 h. The same trend was observed for the algal combination, which in its pure form had a flavonoid content of 467.07 ± 35.62 mg CE/g of sample, which, after co-culture, became 344.39 ± 16.80 mg CE/g of sample and 395.82 ± 13.88 mg CE/g of sample respectively at 24 and 48 h. On the other hand, we found an opposite trend for the co-culture containing the LC extract, as the extract of this alga had a value of 2.60 ± 0.63 mg CE/g of sample, which increased significantly ($p < 0.05$) from 24 h onwards and reached value of 5.73 ± 2.68 mg CE/g of sample (Fig. 4a). The effect produced by the co-culture of *L. plantarum* with the algal extracts was also found in the co-culture involving *L. reuteri*, as the extracts of AN and the algal combination in their pure form had a significantly higher flavonoid content ($p < 0.05$) than that found in the co-culture extracts. On the contrary, the presence of LC leads to an increase in the flavonoid content in the co-culture compared to the extract in its pure form (3.76 ± 0.93 and 2.60 ± 0.63 mg CE/g of the sample, respectively) (Fig. 4b). The flavonoid content in co-cultures of lactobacilli and algae has been the subject of increasing attention from researchers in recent years. However, the data in the literature are still lacking or contradictory (J. Yu et al., 2022; Zhao et al., 2021). In fact, in some studies, co-culture leads to an increase in flavonoid content (Khan et al., 2020), and other studies have shown a decrease in flavonoid content (Le Rouzic et al., 2023). The mechanisms behind this increase are not fully understood. However, this may be linked to the antioxidant effects of lactobacilli and algae (J. Yu et al., 2022), where lactobacilli can protect flavonoids from degradation by free radicals, while algae can provide precursors for the synthesis of flavonoids (Goiris et al., 2014; Piekarska-Radzik & Klewicka, 2021). On the other hand, such mechanisms of action can also be associated with the metabolic effects of lactobacilli, which could use flavonoids as a source of energy, leading to their degradation (He et al., 2022).

3.5. Evaluation of ABTS radical scavenging activity

In recent years, studies on the co-culture of algae and lactobacilli have been conducted to evaluate the benefits of these combinations and their influence on human and animal health. Even if there are few studies on the antioxidant activity demonstrated by these co-cultures, these researches seem promising. A study conducted by Niccolai A (Niccolai et al., 2019) showed that the product derived from the lactic fermentation of *Arthrospira platensis* had a higher antioxidant activity than the non-fermented product. Reboleira J. (Reboleira et al., 2021), focusing his attention on co-cultures between lactobacilli sp. and brown algae, showed an increase in antioxidant activity and phenolic compounds. Further, Dai J. et al. (Dai et al., 2022) showed that in the presence of *Euglena gracilis*, a unicellular microalga, the antioxidant activity of *Lactobacillus* was significantly improved. Our results are in

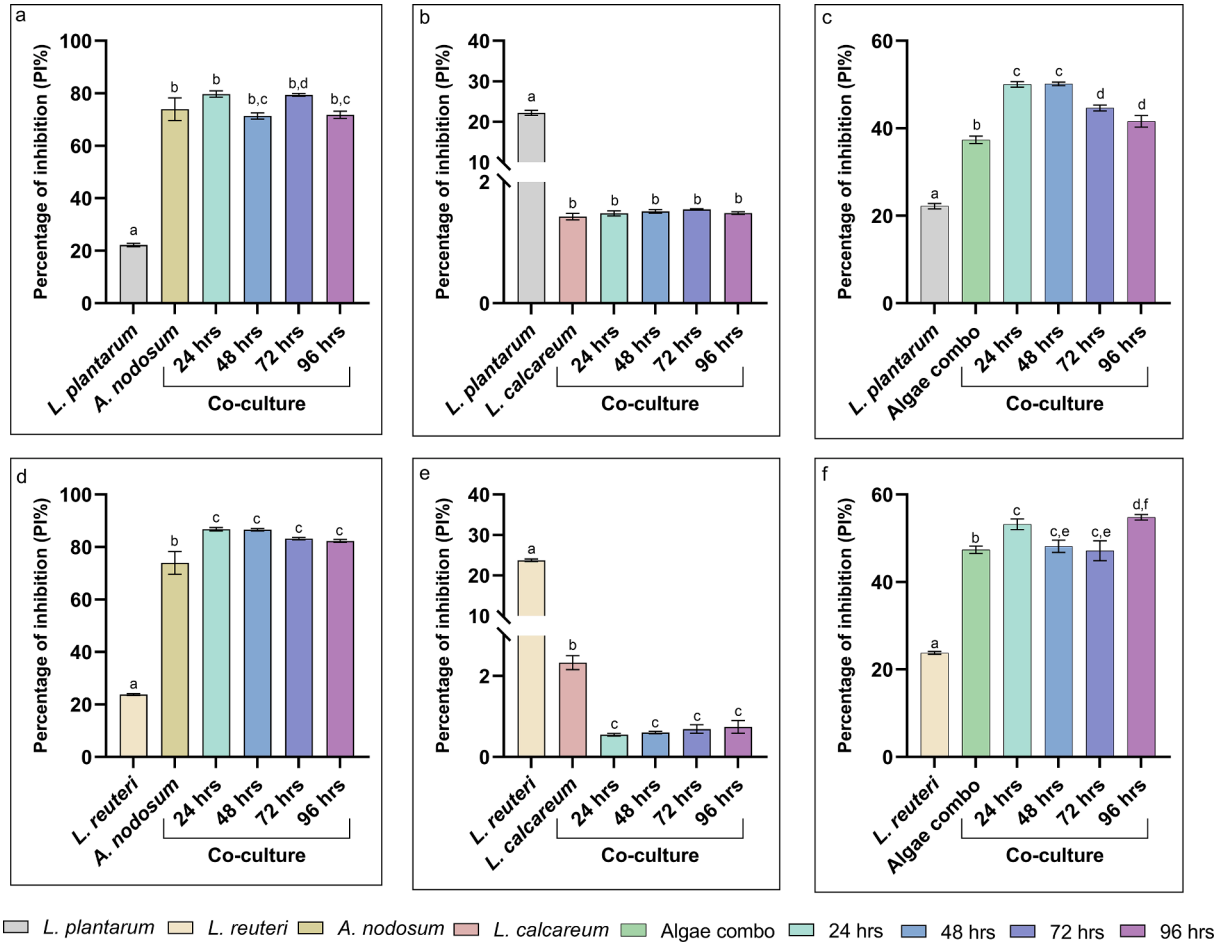

Fig. 5. Percentage of inhibition (PI%) of radical scavenging activity of *Lactiplantibacillus plantarum* and *Limosilactobacillus reuteri* co-culture with algal extracts in different time points of incubation: PI% of L. plantarum cultured with *A. nodosum* (a); PI% of *L. plantarum* cultured with *L. calcareum* (b); PI% of *L. plantarum* cultured with algae combination (c); PI% of *L. reuteri* cultured with *A. nodosum* (d); PI% of *L. reuteri* cultured with *L. calcareum* (e); PI% of *L. reuteri* cultured with algae combination (f). Data are shown as Means ± standard deviations (SD) (n = 3). $^{a, b, c, d, e, f}$ Means with different superscript letters indicate statistically significant differences ($p < 0.05$).

line with what has been reported so far in the literature. The data obtained have displayed that the co-culture carried out with the combination of the selected algal species (AN and LC) and the two species of lactobacilli (*L. plantarum* and *L. reuteri*) have antioxidant activity values higher than both the algal extract and the single culture of *L. plantarum* and *L. reuteri* (Fig. 5c and Fig. 5f). Moreover, in the presence of *L. plantarum*, the co-culture with the algal combination showed its maximum levels of antioxidant activity after 24 and 48 h of incubation (50.05 ± 1.88 PI% and 50.16 ± 1.17 PI%) (Fig. 5c). This peak of activity was delayed to 96 h of incubation (41.60 ± 3.91 PI%) in the co-culture containing *L. reuteri,* probably due to slower growth of the lactic strain (Fig. 5f). The co-cultures containing AN and LC individually lead to a significant increase ($p < 0.001$) in the antioxidant activity value compared to the single culture of *L. plantarum* (22.21 ± 1.89 PI%) but does not lead to significant changes ($p > 0.005$) compared to the antioxidant activity exerted by the AN extract (Fig. 5a). On the contrary, the co-culture of AN with *L. reuteri* showed a significantly higher and constant antioxidant activity value at different incubation time points (85.19 ± 2.65 PI%) both compared to the single culture of *L. reuteri* (23.95 ± 0.97 PI%) and to the single algal extract (73.94 ± 10.57 PI%) (Fig. 5d). Finally, the presence of LC did not increase the antioxidant activity of the co-culture (Fig. 5b and 5e). These results confirmed that algae's presence can increase *Lactobacilli*'s antioxidant activity. Although there are still many studies to be conducted to identify the correct mechanism of action, it is possible to state that *Lactobacillus* can naturally produce antioxidant enzymes, such as superoxide dismutase, an enzyme that converts superoxide, a harmful free radical, into water and oxygen, and catalase, an enzyme that converts hydrogen peroxide, another harmful free radical, always into water and oxygen (Al-Dhabi et al., 2020; George Kerry et al., 2018; Hoffmann et al., 2021; Mu et al., 2018). At the same time, algae can provide antioxidant compounds, such as polyphenols, carotenoids and astaxanthin, helping to protect cells from oxidative damage in a variety of ways, such as by (i) neutralizing free radicals, (ii) protecting cell membranes from damage and (iii) helping to repair damaged cells (Abo-Shady et al., 2023; Frazzini et al., 2022; Sansone & Brunet, 2020). Moreover, *Lactobacilli* and algae can interact with each other to improve antioxidant activity. For example, *Lactobacilli* can produce antioxidant enzymes that can help protect antioxidant compounds in algae from damage. In addition, *Lactobacilli* can also support to improve the absorption of antioxidant compounds in algae (Perković et al., 2022; You et al., 2022). In addition, the obtained results also demonstrated that the two algal species can have a synergistic effect when combined. This effect as also seen in our previous study (Frazzini et al., 2022) may result from the co-presence of different antioxidant molecules and molecular classes. Individually, these components may passes limited antioxidant power, but are able to synergize the bioactive profile of the matrix in question, thus demonstrating a complete bioactive profile (Assadi et al., 2019; Liu et al., 2008; Makanjuola et al., 2015). Nevertheless, with the limited number of studies the precise molecular mechanisms of action that allow the development of a synergistic effect still need to be elucidated by further studies that will investigate deeply the involved molecular pathway.

3.6. Evaluation of antimicrobial activity

To evaluate the antimicrobial capacity of the co-culture of the selected algal species with *L. plantarum* and *L. reuteri*, the growth inhibition of the F18 $^{+}$ strain of *Escherichia coli* was first evaluated, associated with edema disease in pigs and enterotoxaemia. The obtained results showed that the presence of AN in the culture of *L. plantarum* led to significant growth inhibition ($p < 0.005$) from the fourth hour of incubation for the 96-hour-co-culture, while regardless the incubation time point of the co-cultures is observable a significant inhibition capacity after sixth hour of incubation, (Supplementary Fig. 1a). On the other hand, in the co-culture involving *L. reuteri,* significant growth inhibition was observed only for some time point of co-culture. In fact, the results showed that a significant reduction ($p = 0.004$) in the growth of *E. coli* was visible mainly in the sample from the co-culture of 72 h (Supplementary Fig. 1b). Similar results were observed in the co-cultures involving the introduction of LC (Supplementary Fig. 2). In this case, the results showed that when the co-culture was supplemented with *L. plantarum*, the growth inhibition was visible only for the 96-hour co-culture time point, and the values obtained were significantly lower than the value of the growth of *E. coli* only from the fifth hour of incubation (8.45 ± 0.08 Log_{10} Cell/mL vs 8.32 ± 0.02 Log_{10} Cell/mL respectively). In addition to these results, if LC was placed in co-culture with *L. reuteri*, an inhibition effect was observed also for the time point relative to 48 h of co-culture. In this case, the effect was visible since the fourth hour of incubation (8.08 ± 0.17 Log_{10} Cell/mL against 8.29 ± 0.05 Log_{10} Cell/mL of *E. coli*). The inhibition of *E. coli* growth did not change significantly when the algae were placed in combination within the co-cultures. In fact, the decrease in the growth of *E. coli* subjected to these growth conditions (Supplementary Fig. 3), but equally to the results previously illustrated, this effect is visible only in the last hours of incubation. Following the results obtained from the growth inhibition assay, the antimicrobial activity was evaluated by the minimum inhibitory concentration (MIC) assay. The obtained results showed that the presence of algae in the culture of the considered lactobacilli leads to an improvement in antimicrobial activity (Table 2). The minimum concentrations required to induce an antimicrobial effect were lower in the co-cultures than those required for the cultures of the single lactobacilli (18 mg/mL for *Lactiplantibacillus plantarum* and 20 mg/mL for *Limosilactobacillus reuteri*) and the algal extracts (15 mg/mL for *Ascophyllum nodosum and Lithothamnium calcareum*; 13 mg/mL for the combination of algae) to show an antimicrobial effect. Although no studies are present in the literature which evaluate in detail the antimicrobial effects that the co-culture of *Lactobacilli* and algae, these results could be explained by the characteristics and nature of the substances. In fact, on the one hand, *Lactobacilli*, a group of gram-positive, facultative aerobic bacteria, are important for maintaining the intestinal health of living organisms and can also have antimicrobial activity against some pathogenic microorganisms. Their antimicrobial activity is due to several factors, including (i) the production of lactic acid, which creates an acidic environment that inhibits the growth of pathogenic bacteria; (ii) the production of bacteriocins, proteins that can kill or inhibit the growth of other bacteria; (iii) competition for nutrients, which can make it more difficult for pathogenic bacteria to grow and (iv) the induction of immunity, which can help the body fight infections (C.-C. Chen et al., 2019; Radwan & Maryam, 2022). On the other hand, algae, photosynthetic organisms and an important source of nutrients and other natural products, also have antimicrobial activity, inhibiting the growth of pathogenic microorganisms. The antimicrobial activity of algae is due to several factors, including (i) the production of secondary metabolites, such as fatty acids, alcohols and polyphenols, which have antibacterial, antifungal and antiviral properties and (ii) the production of bacteriocins, which are proteins that can kill or inhibit the growth of other bacteria (Abu-Ghannam & Rajauria, 2013; Frazzini et al., 2022; Shannon & Abu-Ghannam, 2016). Therefore, although the mechanisms underlying the antimicrobial activity of the co-culture of lactobacilli and algae are not fully elicited, it is likely that the antimicrobial metabolites produced by both microorganisms interact to enhance antimicrobial activity.

4. Conclusion

In conclusion, our study demonstrates that when *Lactobacillus* bacteria are cultured alongside algae, they exhibit a synergistic effect, showcasing potential benefits for various industries, especially in nutrition and health. The research confirms that incorporating algae into *Lactobacillus* cultures is safe and may even enhance the bacteria beneficial properties. In fact, the combination of algae and *Lactobacillus* not only hinder bacterial growth but actually enhanced their antioxidant

Table 2
Minimal inhibitory concentration (MIC) of *Lactiplantibacillus plantarum* and *Limosilactobacillus reuteri* co-culture with algal extracts against *E. coli* F18^{+} in different time points of incubation.

	Extract concentration (mg/mL)					
	Lactiplantibacillus plantarum			*Limosilactobacillus reuteri*		
	A. nodosum	*L. calcareum*	Combination	*A. nodosum*	*L. calcareum*	Combination
24hrs culture	25	20	10	15	20	15
48hrs culture	10	10	5	10	20	10
72hrs culture	7	8	5	5	15	1
96hrs culture	4	7	5	5	8	1
	Inhibition rate (%)					
	Lactiplantibacillus plantarum			*Limosilactobacillus reuteri*		
	A. nodosum	*L. calcareum*	Combination	*A. nodosum*	*L. calcareum*	Combination
24hrs culture	30.81	43.33	59.57	53.21	32.96	67.88
48hrs culture	65.45	58.40	78.11	73.34	61.92	79.97
72hrs culture	62.57	79.85	87.76	86.51	36.64	95.88
96hrs culture	62.39	42.86	31.41	28.05	19.22	76.33

and antimicrobial activities, indicating a mutually beneficial interaction. This suggests that certain algae species, such as *Ascophyllum nodosum* and *Lithothamnium calcareum*, may be utilized as prebiotics without impeding *Lactobacillus* growth. This opens up new possibilities for applying algae as prebiotics to enhance health. The findings have significant implications for industries involved in animal and human nutrition, offering innovative strategies for diet formulation. However, the study acknowledges the necessity for further research to understand the underlying mechanisms and explore additional applications of this synergistic effects.

process.

Ethical statement

The authors state that neither animals nor humans were used for this study.

Author contributions

Conceptualisation: **Sara Frazzini**, **Luciana Rossi**; Methodology: **Sara Frazzini, Maria Claudia Torresani**; Investigation: **Sara Frazzini, Maria Claudia Torresani**; Data curation: **Sara Frazzini**; Writing original draft preparation: **Sara Frazzini**; Writing review and editing: **Sara Frazzini, Monika Hejna, Luciana Rossi**; Visualisation: **Sara Frazzini**; Supervision: **Monika Hejna**, **Michele Di Dio, Luciana Rossi**; Funding acquisition: **Luciana Rossi**.

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Declaration of Generative AI and AI-assisted technologies in the writing process

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CRediT authorship contribution statement

Sara Frazzini: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Data curation, Conceptualization. **Maria Claudia Torresani:** Methodology, Investigation. **Monika Hejna:** Writing – review & editing, Supervision. **Michele Di Dio:** Supervision. **Luciana Rossi:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jff.2024.106257.

References

Abo-Shady, A. M., Gheda, S. F., Ismail, G. A., Cotas, J., Pereira, L., & Abdel-Karim, O. H. (2023). Antioxidant and Antidiabetic Activity of Algae. *Life*, *13*(2), 460. doi: 0.3390/life13020460.

Abu-Ghannam, N., & Rajauria, G. (2013). Antimicrobial activity of compounds isolated from algae. In *Functional Ingredients from Algae for Foods and Nutraceuticals* (pp. 287–306). Elsevier. doi: 0.1533/9780857098689.2.287.

Adebo, O. A., & Gabriela Medina-Meza, I. (2020). Impact of Fermentation on the Phenolic Compounds and Antioxidant Activity of Whole Cereal Grains: A Mini Review. *Molecules*, *25*(4), 927. doi: 0.3390/molecules25040927.

Adefegha, S. A. (2018). Functional Foods and Nutraceuticals as Dietary Intervention in Chronic Diseases; Novel Perspectives for Health Promotion and Disease Prevention. *Journal of Dietary Supplements, 15*(6), 977–1009. https://doi.org/10.1080/19390211.2017.1401573

Al-Dhabi, N. A., Valan Arasu, M., Vijayaraghavan, P., Esmail, G. A., Duraipandiyan, V., Kim, Y. O., Kim, H., & Kim, H.-J. (2020). Probiotic and Antioxidant Potential of Lactobacillus reuteriLR12 and Lactobacillus lactisLL10 Isolated from Pineapple Puree and Quality Analysis of Pineapple-Flavored Goat Milk Yoghurt during Storage. *Microorganisms, 8*(10), 1461. https://doi.org/10.3390/microorganisms8101461

Alexandratos, N., & Bruinsma J. (2012). *World agriculture towards 2030/2050: the 2012 revision.* (12; 03).

Almeida, F., Schiavo, L. V., Vieira, A. D., Araújo, G. L., Queiroz-Junior, C. M., Teixeira, M. M., Cassali, G. D., & Tagliati, C. A. (2012). Gastroprotective and toxicological evaluation of the Lithothamnion calcareum algae. *Food and Chemical Toxicology, 50*(5), 1399–1404. https://doi.org/10.1016/j.fct.2012.02.028

Assadi, I., Elfalleh, W., Benabderrahim, M. A., Hannachi, H., Chaalen, W., & Ferchichi, A. (2019). Nutritional Quality and Antioxidant Capacity of a Combination of Pomegranate and Date Juices. *International Journal of Fruit Science, 19*(3), 300–314. https://doi.org/10.1080/15538362.2018.1512438

Burel, C., Kala, A., & Purevdorj-Gage, L. (2021). Impact of pH on citric acid antimicrobial activity against Gram-negative bacteria. *Letters in Applied Microbiology, 72*(3), 332–340. https://doi.org/10.1111/lam.13420

Cantú-Bernal, S., Domínguez-Gámez, M., Medina-Peraza, I., Aros-Uzarraga, E., Ontiveros, N., Flores-Mendoza, L., Gomez-Flores, R., Tamez-Guerra, P., & González-Ochoa, G. (2020). Enhanced Viability and Anti-rotavirus Effect of Bifidobacterium longum and Lactobacillus plantarum in Combination With Chlorella sorokiniana in a Dairy Product. *Frontiers in Microbiology, 11*. https://doi.org/10.3389/fmicb.2020.00875

Chapman, R. L. (2013). Algae: The world's most important "plants"—an introduction. *Mitigation and Adaptation Strategies for Global Change, 18*(1), 5–12. https://doi.org/10.1007/s11027-010-9255-9

Chen, C.-C., Lai, C.-C., Huang, H.-L., Huang, W.-Y., Toh, H.-S., Weng, T.-C., Chuang, Y.-C., Lu, Y.-C., & Tang, H.-J. (2019). Antimicrobial Activity of Lactobacillus Species Against Carbapenem-Resistant Enterobacteriaceae. *Frontiers in Microbiology, 10*. https://doi.org/10.3389/fmicb.2019.00789

Chen, P.-T., Hong, Z.-S., Cheng, C.-L., Ng, I.-S., Lo, Y.-C., Nagarajan, D., & Chang, J.-S. (2020). Exploring fermentation strategies for enhanced lactic acid production with polyvinyl alcohol-immobilized Lactobacillus plantarum 23 using microalgae as

feedstock. *Bioresource Technology, 308*, Article 123266. https://doi.org/10.1016/j.biortech.2020.123266
Dai, J., He, J., Chen, Z., Qin, H., Du, M., Lei, A., Zhao, L., & Wang, J. (2022). Euglena gracilis Promotes Lactobacillus Growth and Antioxidants Accumulation as a Potential Next-Generation Prebiotic. *Frontiers in Nutrition, 9*. https://doi.org/10.3389/fnut.2022.864565
de Jesus Raposo, M., de Morais, A., & de Morais, R. (2015). Marine Polysaccharides from Algae with Potential Biomedical Applications. *Marine Drugs, 13*(5), 2967–3028. https://doi.org/10.3390/md13052967
Dell'Anno, M., Giromini, C., Reggi, S., Cavalleri, M., Moscatelli, A., Onelli, E., Rebucci, R., Sundaram, T. S., Coranelli, S., Spalletta, A., Baldi, A., & Rossi, L. (2021). Evaluation of Adhesive Characteristics of L. *plantarum and L. reuteri Isolated from Weaned Piglets. Microorganisms, 9*(8), 1587. https://doi.org/10.3390/microorganisms9081587
Efsa. (2012). Guidance on the assessment of bacterial susceptibility to antimicrobials of human and veterinary importance. *EFSA Journal, 10*(6). https://doi.org/10.2903/j.efsa.2012.2740
Commission, E. (2019). Regulation (EU) 2019/6 of the European Parliament and of the Council of 11 December 2018 on veterinary medicinal products and repealing Directive 2001/82/EC. *Official Journal of the European Union*.
Fao. (2019). World Livestock: Transforming the livestock sector through the Sustainable Development Goals. *FAO*. https://doi.org/10.4060/ca1201en
Frazzini, S., Scaglia, E., Dell'Anno, M., Reggi, S., Panseri, S., Giromini, C., Lanzoni, D., Sgoifo Rossi, C. A., & Rossi, L. (2022). Antioxidant and Antimicrobial Activity of Algal and Cyanobacterial Extracts: An In Vitro Study. *Antioxidants, 11*(5), 992. https://doi.org/10.3390/antiox11050992
Frazzini, S., Zuorro, A., Panseri, S., Pavlovic, R., Sgoifo Rossi, C. A., & Rossi, L. (2023). Repurposing Hazelnut Waste Products for a Sustainable Economy: A Metabolomic Analysis of Cuticles and Shells to Highlight Their Antioxidant Potential and Inhibitory Activity against Verocytotoxic Escherichia coli. *Sustainability, 15*(4), 3268. https://doi.org/10.3390/su15043268
George Kerry, R., Patra, J. K., Gouda, S., Park, Y., Shin, H.-S., & Das, G. (2018). Benefaction of probiotics for human health: A review. *Journal of Food and Drug Analysis, 26*(3), 927–939. https://doi.org/10.1016/j.jfda.2018.01.002
Giraffa, G., Chanishvili, N., & Widyastuti, Y. (2010). Importance of lactobacilli in food and feed biotechnology. *Research in Microbiology, 161*(6), 480–487. https://doi.org/10.1016/j.resmic.2010.03.001
Goiris, K., Muylaert, K., Voorspoels, S., Noten, B., De Paepe, D., Baart, E. G. J., & De Cooman, L. (2014). Detection of flavonoids in microalgae from different evolutionary lineages. *Journal of Phycology, 50*(3), 483–492. https://doi.org/10.1111/jpy.12180
Gouveia, L., Batista, A. P., Sousa, I., Raymundo, A., & Bandarra, N. M. (2008). *Microalgae in novel food products*. Nova Science Publishers.
Gupta, S., & Abu-Ghannam, N. (2011). Recent developments in the application of seaweeds or seaweed extracts as a means for enhancing the safety and quality attributes of foods. *Innovative Food Science & Emerging Technologies, 12*(4), 600–609. https://doi.org/10.1016/j.ifset.2011.07.004
Hadj Saadoun, J., Bertani, G., Levante, A., Vezzosi, F., Ricci, A., Bernini, V., & Lazzi, C. (2021). Fermentation of Agri-Food Waste: A Promising Route for the Production of Aroma Compounds. *Foods, 10*(4), 707. https://doi.org/10.3390/foods10040707
Harinder P. S. Makkar, & Philippe Ankers. (2014). *Towards a concept of Sustainable Animal Diets*.
He, Z., Zhang, H., Wang, T., Wang, R., & Luo, X. (2022). Effects of Five Different Lactic Acid Bacteria on Bioactive Components and Volatile Compounds of Oat. *Foods, 11*(20), 3230. https://doi.org/10.3390/foods11203230
Heleno, S. A., Ferreira, I. C. F. R., Esteves, A. P., Ćirić, A., Glamočlija, J., Martins, A., Soković, M., & Queiroz, M. J. R. P. (2013). Antimicrobial and demelanizing activity of Ganoderma lucidum extract, p-hydroxybenzoic and cinnamic acids and their synthetic acetylated glucuronide methyl esters. *Food and Chemical Toxicology, 58*, 95–100. https://doi.org/10.1016/j.fct.2013.04.025
Heleno, S. A., Martins, A., Queiroz, M. J. R. P., & Ferreira, I. C. F. R. (2015). Bioactivity of phenolic acids: Metabolites versus parent compounds: A review. *Food Chemistry, 173*, 501–513. https://doi.org/10.1016/j.foodchem.2014.10.057
Herald, T. J., Gadgil, P., & Tilley, M. (2012). High-throughput micro plate assays for screening flavonoid content and DPPH-scavenging activity in sorghum bran and flour. *Journal of the Science of Food and Agriculture, 92*(11), 2326–2331. https://doi.org/10.1002/jsfa.5633
Hoffmann, A., Kleniewska, P., & Pawliczak, R. (2021). Antioxidative activity of probiotics. *Archives of Medical Science, 17*(3), 792–804. https://doi.org/10.5114/aoms.2019.89894
Holdt, S. L., & Kraan, S. (2011). Bioactive compounds in seaweed: Functional food applications and legislation. *Journal of Applied Phycology, 23*(3), 543–597. https://doi.org/10.1007/s10811-010-9632-5
Iorizzo, M., Testa, B., Ganassi, S., Lombardi, S. J., Ianiro, M., Letizia, F., Succi, M., Tremonte, P., Vergalito, F., Cozzolino, A., Sorrentino, E., Petrarca, S., De Cristofaro, A., & Coppola, R. (2021). Probiotic Properties and Potentiality of Lactiplantibacillus plantarum Strains for the Biological Control of Chalkbrood Disease. *Journal of Fungi, 7*(5), 379. https://doi.org/10.3390/jof7050379
Jamnik, P., Mahnič, N., Mrak, A., Pogačnik, L., Jeršek, B., Niccolai, A., Masten Rutar, J., Ogrinc, N., Dušak, L., Ferjančič, B., Korošec, M., Cerar, A., Lazar, B., Lovše, U., Pungert, T., Fabjan, P., & Poklar Ulrih, N. (2022). Fermented Biomass of Arthrospira platensis as a Potential Food Ingredient. *Antioxidants, 11*(2), 216. https://doi.org/10.3390/antiox11020216
Khan, S. A., Zhang, M., Liu, L., Dong, L., Ma, Y., Wei, Z., Chi, J., & Zhang, R. (2020). Co-culture submerged fermentation by lactobacillus and yeast more effectively improved the profiles and bioaccessibility of phenolics in extruded brown rice than single-culture fermentation. *Food Chemistry, 326*, Article 126985. https://doi.org/10.1016/j.foodchem.2020.126985
Khemiri, S., Khelifi, N., Messaoud, C., & Smaali, I. (2023). Bioprospecting of microalgae for a potential use as enzyme inhibitors, anti-ageing and prebiotic agents. *Biocatalysis and Agricultural Biotechnology, 51*, Article 102759. https://doi.org/10.1016/j.bcab.2023.102759
Le Rouzic, M., Bruniaux, P., Raveschot, C., Krier, F., Phalip, V., Ravallec, R., Cudennec, B., & Coutte, F. (2023). *Lactobacillus* Use for Plant Fermentation: New Ways for Plant-Based Product Valorization. In *Lactobacillus - A Multifunctional Genus*. IntechOpen. doi: 0.5772/intechopen.104958.
Li, Z., Dong, Y., Zhang, Y., Zheng, M., Jiang, Z., Zhu, Y., Deng, S., Li, Q., & Ni, H. (2023). Lactobacillus-fermentation enhances nutritional value and improves the inhibition on pancreatic lipase and oral pathogens of edible red seaweed Bangia fusco-purpurea. *LWT, 179*, Article 114643. https://doi.org/10.1016/j.lwt.2023.114643
Liu, D., Shi, J., Colina Ibarra, A., Kakuda, Y., & Jun Xue, S. (2008). The scavenging capacity and synergistic effects of lycopene, vitamin E, vitamin C, and β-carotene mixtures on the DPPH free radical. *LWT - Food Science and Technology, 41*(7), 1344–1349. https://doi.org/10.1016/j.lwt.2007.08.001
Lobo, V., Patil, A., Phatak, A., & Chandra, N. (2010). Free radicals, antioxidants and functional foods: Impact on human health. *Pharmacognosy Reviews, 4*(8), 118. https://doi.org/10.4103/0973-7847.70902
Makanjuola, S. A., Enujiugha, V. N., Omoba, O. S., & Sanni, D. M. (2015). Combination of Antioxidants from Different Sources Could Offer Synergistic Benefits: A Case Study of Tea and Ginger Blend. *Nat Prod Commun, 10*(11), 1829–1832.
Markowiak, P., & Śliżewska, K. (2018). The role of probiotics, prebiotics and synbiotics in animal nutrition. *Gut Pathogens, 10*(1), 21. https://doi.org/10.1186/s13099-018-0250-0
Marques, C., Sotiles, A. R., Farias, F. O., Oliveira, G., Mitterer-Daltoé, M. L., & Masson, M. L. (2020). Full physicochemical characterization of malic acid: Emphasis in the potential as food ingredient and application in pectin gels. *Arabian Journal of Chemistry, 13*(12), 9118–9129. https://doi.org/10.1016/j.arabjc.2020.10.036
Moon, K. M., Kwon, E.-B., Lee, B., & Kim, C. Y. (2020). Recent Trends in Controlling the Enzymatic Browning of Fruit and Vegetable Products. *Molecules, 25*(12), 2754. https://doi.org/10.3390/molecules25122754
Mu, G., Gao, Y., Tuo, Y., Li, H., Zhang, Y., Qian, F., & Jiang, S. (2018). Assessing and comparing antioxidant activities of lactobacilli strains by using different chemical and cellular antioxidant methods. *Journal of Dairy Science, 101*(12), 10792–10806. https://doi.org/10.3168/jds.2018-14989
Myers, J. A., Curtis, B. S., & Curtis, W. R. (2013). Improving accuracy of cell and chromophore concentration measurements using optical density. *BMC Biophysics, 6*(1), 4. https://doi.org/10.1186/2046-1682-6-4
Niccolai, A., Shannon, E., Abu-Ghannam, N., Biondi, N., Rodolfi, L., & Tredici, M. R. (2019). Lactic acid fermentation of Arthrospira platensis (spirulina) biomass for probiotic-based products. *Journal of Applied Phycology, 31*(2), 1077–1083. https://doi.org/10.1007/s10811-018-1602-3
Parracho, H., McCartney, A. L., & Gibson, G. R. (2007). Probiotics and prebiotics in infant nutrition. *Proceedings of the Nutrition Society, 66*(3), 405–411. https://doi.org/10.1017/S0029665107005678
Patel, A. K., Singhania, R. R., Awasthi, M. K., Varjani, S., Bhatia, S. K., Tsai, M.-L., Hsieh, S.-L., Chen, C.-W., & Dong, C.-D. (2021). Emerging prospects of macro- and microalgae as prebiotic. *Microbial Cell Factories, 20*(1), 112. https://doi.org/10.1186/s12934-021-01601-7
Perković, L., Djedović, E., Vujović, T., Baković, M., Paradžik, T., & Čož-Rakovac, R. (2022). Biotechnological Enhancement of Probiotics through Co-Cultivation with Algae: Future or a Trend? *Marine Drugs, 20*(2), 142. https://doi.org/10.3390/md20020142
Piekarska-Radzik, L., & Klewicka, E. (2021). Mutual influence of polyphenols and Lactobacillus spp. bacteria in food: A review. *European Food Research and Technology, 247*(1), 9–24. https://doi.org/10.1007/s00217-020-03603-y
Pugazhendhi, D., Pope, G. S., & Darbre, P. D. (2005). Oestrogenic activity ofp-hydroxybenzoic acid (common metabolite of paraben esters) and methylparaben in human breast cancer cell lines. *Journal of Applied Toxicology, 25*(4), 301–309. https://doi.org/10.1002/jat.1066
Radwan, R. M., & Maryam, R. M. (2022). Antimicrobial Activity of Probiotic Lactobacilli against Some Pathogenic Bacteria. *J Prob Health, 10*(284).
Raposo, M., De Morais, R., & Bernardo de Morais, A. (2013). Bioactivity and Applications of Sulphated Polysaccharides from Marine Microalgae. *Marine Drugs, 11*(1), 233–252. https://doi.org/10.3390/md11010233
Raven, J. A., & Giordano, M. (2014). Algae. *Current Biology, 24*(13), R590–R595. https://doi.org/10.1016/j.cub.2014.05.039
Reboleira, J., Silva, S., Chatzifragkou, A., Niranjan, K., & Lemos, M. F. L. (2021). Seaweed fermentation within the fields of food and natural products. *Trends in Food Science & Technology, 116*, 1056–1073. https://doi.org/10.1016/j.tifs.2021.08.018
Ricci, A., Cirlini, M., Calani, L., Bernini, V., Neviani, E., Del Rio, D., Galaverna, G., & Lazzi, C. (2019). In vitro metabolism of elderberry juice polyphenols by lactic acid bacteria. *Food Chemistry, 276*, 692–699. https://doi.org/10.1016/j.foodchem.2018.10.046
Rybczyńska-Tkaczyk, K., Grenda, A., Jakubczyk, A., Kiersnowska, K., & Bik-Małodzińska, M. (2023). Natural Compounds with Antimicrobial Properties in Cosmetics. *Pathogens, 12*(2), 320. https://doi.org/10.3390/pathogens12020320
Sansone, C., & Brunet, C. (2020). *Marine Algal Antioxidants. Antioxidants, 9*(3), 206. https://doi.org/10.3390/antiox9030206
Ścieszka, S., & Klewicka, E. (2020). Influence of the Microalga Chlorella vulgaris on the Growth and Metabolic Activity of Lactobacillus spp. *Bacteria. Foods, 9*(7), 959. https://doi.org/10.3390/foods9070959

Shakya, S., Danshiitsoodol, N., Sugimoto, S., Noda, M., & Sugiyama, M. (2021). Anti-Oxidant and Anti-Inflammatory Substance Generated Newly in Paeoniae Radix Alba Extract Fermented with Plant-Derived Lactobacillus brevis 174A. *Antioxidants, 10*(7), 1071. https://doi.org/10.3390/antiox10071071

Shannon, E., & Abu-Ghannam, N. (2016). Antibacterial Derivatives of Marine Algae: An Overview of Pharmacological Mechanisms and Applications. *Marine Drugs, 14*(4), 81. https://doi.org/10.3390/md14040081

Terpou, A., Papadaki, A., Lappa, I., Kachrimanidou, V., Bosnea, L., & Kopsahelis, N. (2019). Probiotics in Food Systems: Significance and Emerging Strategies Towards Improved Viability and Delivery of Enhanced Beneficial Value. *Nutrients, 11*(7), 1591. https://doi.org/10.3390/nu11071591

Trabelsi, I., Bejar, W., Ayadi, D., Chouayekh, H., Kammoun, R., Bejar, S., & Ben Salah, R. (2013). Encapsulation in alginate and alginate coated-chitosan improved the survival of newly probiotic in oxgall and gastric juice. *International Journal of Biological Macromolecules, 61*, 36–42. https://doi.org/10.1016/j.ijbiomac.2013.06.035

UN General Assembly. (2015, October 21). *Transforming our world: the 2030 Agenda for Sustainable Development*. https://www.refworld.org/docid/57b6e3e44.html.

Van Den Berg, A. J. J., Halkes, S. B. A., Quarles Van Ufford, H. C., Hoekstra, M. J., & Beukelman, C. J. (2003). A novel formulation of metal ions and citric acid reduces reactive oxygen species *in vitro*. *Journal of Wound Care, 12*(10), 413–418. https://doi.org/10.12968/jowc.2003.12.10.26552

Vidanarachchi, J. K., Kurukulasuriya, M. S., Malshani Samaraweera, A., & Silva, K. F. S. T. (2012). *Applications of Marine Nutraceuticals in Dairy Products* (pp. 457–478). doi: 10.1016/B978-0-12-416003-3.00030-5.

Wang, Y., Compaoré-Sérémé, D., Sawadogo-Lingani, H., Coda, R., Katina, K., & Maina, N. H. (2019). Influence of dextran synthesized in situ on the rheological, technological and nutritional properties of whole grain pearl millet bread. *Food Chemistry, 285*, 221–230. https://doi.org/10.1016/j.foodchem.2019.01.126

Wang, Z., Wu, J., Tian, Z., Si, Y., Chen, H., & Gan, J. (2022). The Mechanisms of the Potential Probiotic Lactiplantibacillus plantarum against Cardiovascular Disease and the Recent Developments in its Fermented Foods. *Foods, 11*(17), 2549. https://doi.org/10.3390/foods11172549

You, S., Ma, Y., Yan, B., Pei, W., Wu, Q., Ding, C., & Huang, C. (2022). The promotion mechanism of prebiotics for probiotics: A review. *Frontiers in Nutrition, 9*. https://doi.org/10.3389/fnut.2022.1000517

Yu, J., Geng, Y., Xia, H., Ma, D., Liu, C., Wu, R., Wu, J., You, S., & Bi, Y. (2022). LAB Fermentation Improves Production of Bioactive Compounds and Antioxidant Activity of *Withania somnifera* Extract and Its Metabolic Signatures as Revealed by LC-MS/MS. *Journal of Microbiology and Biotechnology, 32*(4), 473–483. https://doi.org/10.4014/jmb.2111.11018

Yu, Z., Chen, J., Liu, Y., Meng, Q., Liu, H., Yao, Q., Song, W., Ren, X., & Chen, X. (2023). The role of potential probiotic strains Lactobacillus reuteri in various intestinal diseases: New roles for an old player. *Frontiers in Microbiology, 14*. https://doi.org/10.3389/fmicb.2023.1095555

Zaporozhets, T. S., Besednova, N. N., Kuznetsova, T. A., Zvyagintseva, T. N., Makarenkova, I. D., Kryzhanovsky, S. P., & Melnikov, V. G. (2014). The prebiotic potential of polysaccharides and extracts of seaweeds. *Russian Journal of Marine Biology, 40*(1), 1–9. https://doi.org/10.1134/S1063074014010106

Zhao, Y.-S., Eweys, A. S., Zhang, J.-Y., Zhu, Y., Bai, J., Darwesh, O. M., Zhang, H.-B., & Xiao, X. (2021). Fermentation Affects the Antioxidant Activity of Plant-Based Food Material through the Release and Production of Bioactive Components. *Antioxidants, 10*(12), 2004. https://doi.org/10.3390/antiox10122004

Zhong, B., Robinson, N. A., Warner, R. D., Barrow, C. J., Dunshea, F. R., & Suleria, H. A. R. (2020). LC-ESI-QTOF-MS/MS Characterization of Seaweed Phenolics and Their Antioxidant Potential. *Marine Drugs, 18*(6), 331. https://doi.org/10.3390/md18060331