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Fermentability of *Opuntia ficus-indica* cladodes powder by the probiotic *Lactiplantibacillus plantarum* F2: First insights on the strengthening of both health- beneficial effects

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Abstract. *Opuntia ficus-indica* is a succulent plant of *Cactaceae* family, easily distinguished by the presence of characterized branches called "cladodes". The latter are rich in bioactive components such as dietary fibers, vitamins and polyphenols, and thus could be considered as a functional food or food ingredient. The main objectives of this study are (a) to investigate the fermentability of *Opuntia ficus-indica* cladodes by a probiotic strain *Lactiplantibacillus plantarum* F2, (b) to determine the effect of the cladodes on the beneficial effects of the probiotic strain, and (c) to examine the influence of the plant age, sterilization method and the concentration on the observed effects. For this, nine concentrations of either young (3-6 months old) or aged (>1-year-old) cladodes were tested. Firstly, the cladodes were examined on their physicochemical characteristics. Then, their effect on bacterial growth, surface properties and adhesion, antibacterial and anti-biofilm activities and antioxidant and cholesterol lowering potentials were investigated. The results showed an enhancement in growth and strengthening of the probiotic strain performances (higher antibacterial, adhesion, anti-biofilm, antioxidant and cholesterol lowering activities). The effects of the powder were recorded in age-, sterilization method and concentration - dependent manners. In general, most pronounced effects were observed with young cladodes, in filtered macerates and at high concentrations. Nevertheless, opposite observation was noted for the antioxidant potential, which was better in the presence of aged cladodes. In whole the results gathered in this work showed a synergistic effect between the probiotic strain and the *O. ficus-indica* cladodes macerate.

Keywords: *Opuntia ficus-indica, cladodes, Lactiplantibacillus plantarum, prebiotic effect, antibacterial activity, adhesion.*

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Introduction

Opuntia ficus-indica is a member of the *Cactaceae* family that grows in many regions such as Mexico, North and South America, Australia, Africa, and the Mediterranean area, including Algeria (Felkai-Haddache *et al*., 2016a). The cactus plantation in Algeria covers about 28,000 hectares in 2015 (Felkai-Haddache *et al*., 2016b). *Opuntia ficus-indica* (L.) Mill is among the highly abundant cacti in Algeria, characterized by its spineless cladodes (Mahdeb *et al.*, 2021). These vegetal structures are rich in many bioactive compounds such dietary fibers that are known

for their capacity to improve digestion and to regulate intestinal transit. The term “dietary fiber” was used for the first time in 1953 to describe the components of the plant cell wall that was not digestible by humans (Devries *et al*., 1999). Insoluble fibers play the role of maintaining the regulation of intestinal function, modulating peristalsis, controlling pH, and contributing to the prevention of gastrointestinal diseases (Weickert and Pfeiffer, 2008). In the colon, they are fermented by microbiome, allowing the dominance of beneficial bacterial species, such as lactobacilli and bifidobacteria, thus classifying these products as prebiotics (Cummings *et al.*, 2001). Soluble fibers possess hypoglycemic, hypolipidemic, and hypocholesterolemic properties (Peña-Valdivia *et al*., 2012). They form a viscous gel in the intestine, which slows digestion, potentially leading to better blood sugar control and reduced hunger, and improves the balance of the microbiome (Cruz-Rubio *et al.*, 2020) and thus could also be considered as prebiotics. In addition, the bioactive compounds contained in *O. ficus-indica* justify many of the beneficial activities carried out by this plant on human health, such as antioxidant, anti-inflammatory, anticancer, antimicrobial, and nutritional properties (Caminiti *et al*., 2024). Thanks to their fibers content, cladodes are highly functional, which increases their market value and makes them important promoters of nutritional properties and prized in medical, cosmetic, and pharmaceutical industries (Rodrigues *et al*., 2023). The prebiotic concept was largely defined in the literature and one of the first descriptions is that proposed in 1995 by Gibson and Roberfroid (Gibson *et al.*, 2004). Nowadays, the International Scientific Association for Probiotics and Prebiotics (ISAPP) defines a prebiotic as a substrate that is selectively utilized by host microorganisms, conferring a health benefit (ISAPP, 2016). According to Laparra and Sanz (2010), three criteria are mandatory for measuring the efficiency of a prebiotic: (*i*) resistance to stomach acidity and the digestive enzymes, (*ii*) possibility to be fermented by the intestinal micro-organisms and, (*iii*) modulation and stimulation of the beneficial bacteria growth in the intestine. In parallel to prebiotics, probiotics are live microorganisms that when administered in adequate amounts exert beneficial effects on the host, generally by balancing the intestinal microflora (FAO/WHO, 2002). It is widely recognized that probiotics and prebiotics have significant effects in prevention and treatment of gastrointestinal disorders and the subsequent intestinal diseases like irritable colon syndrome and inflammatory bowel disease (Jinkins and Mason, 2022; Ali *et al*., 2025). In Algeria, few studies were conducted on *Opuntia* sp. cladodes and most of them deals with the mucilage, pectin and phenolic compounds extraction and characterization (Felkai-Haddache *et al*., 2016 *a, b*; Adjeroud *et al*., 2015, 2018; Djerroud *et al.*, 2017; Lefsih *et al*., 2018; Mahdeb *et al*., 2021; Adjeroud-Abdellatif *et al.*, 2022). On the other hand, some studies were focused on *Opuntia* sp. fruit (Mazari *et al*., 2018; Boudjouan *et al*., 2022). To our knowledge, no study was conducted on the prebiotic effects of *Opuntia* sp. cladodes. So, the present study fills this gap by investigating the effect of *O. ficus-indica* cladodes on the bacterial growth and probiotic features of *Lactiplantibacillus plantarum* F2, a newly isolated and characterized probiotic strain.

Material and Methods

Opuntia ficus-indica cladodes collection

The cladodes were collected on September 15th, 2023, in the National Institute of Agronomic Research of Algeria (INRAA), Oued-Ghir station (Bejaia city) (Figure 1). Two cladode types were collected, old cladodes (age > 1 year) and young cladodes (age = 3-6 months). The sampling period coincided with a dry period, which explains their high dryness (Figure 2). The collected cladodes were first washed with distilled water containing a disinfectant, dried on air, then cut on small pieces and introduced in an aerated oven for 48 h at 65 °C. The completely dry samples obtained were crushed in a blender; the generated powder (P1 for young cladodes and P2 for aged cladodes) was sifted using a 250 µm

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mesh size sieve. The powder products (P1 and P2) were kept in a dried, hermetically well-sealed glass container at −20 °C until use.

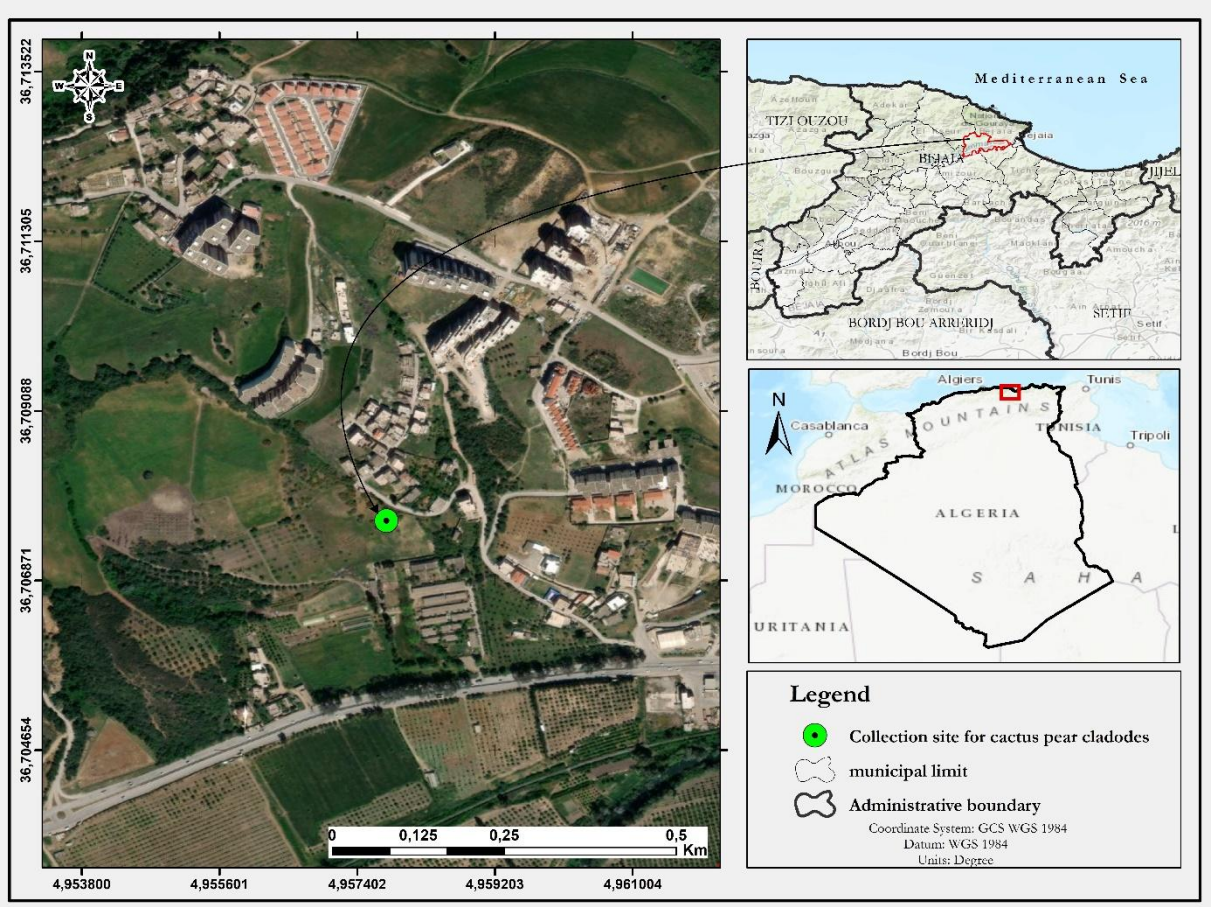

Figure 1. Sampling map of the *Opuntia ficus-indica* cladodes.

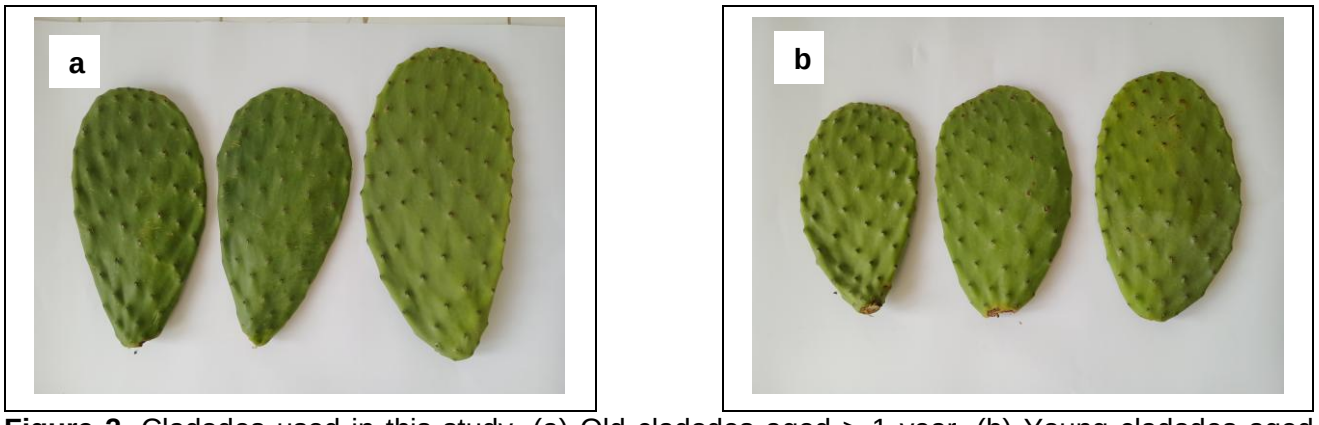

Figure 2. Cladodes used in this study, (a) Old cladodes aged > 1 year, (b) Young cladodes aged between 03 and 06 months.

Physicochemical analyses of the cladodes

Water and mineral content

Water content of the cladodes was determined by the method 925.10 of the Association of Official Analytical Chemists (AOAC, 2000). For this, 2 g of cladodes were desiccated in an aerated oven at 105 °C until constant weight. The difference in weight before and after drying is used to calculate the water content. The mineral content, expressed in % of dry matter (DM), was determined by mineralization of 2 g of the cladodes dry matter, at 550 °C overnight according to the method 942.05 (AOAC, 2000). For determining the contents of calcium (Ca), potassium (K), and magnesium (Mg), the ash samples were solubilized with nitric acid, filtered through ash-free-filter paper, and then put into 100 mL flasks that had been filled with demineralized water up to the mark. The analysis of minerals (expressed in mg/100 g DM) was performed by atomic absorption spectrometry (Hitachi Z6100, Tokyo, Japan).

Total sugar and crude fiber content

Total neutral sugars

The total sugar content was determined using the phenol-sulfuric acid method (Dubois *et al*., 1956). Sugar extraction was performed following the AOAC (2000) method. Briefly, 0.1 g of sample was mixed with 30 mL of 80% (v/v) ethanol and allowed to stand for 48 h at room temperature. After removing the ethanol by evaporation at 80 °C, 20 mL of distilled water was added to the extract to obtain the solution for analysis. Then, 1 mL of 5% (v/v) phenol solution was added to 1 mL of the solution to be analyzed. After stirring, 5 mL of concentrated sulfuric acid (98%) were added, and the stirring was continued. After standing, the solution was put in a water bath at 30 °C/15 min and then cooled. After 30 min, the absorbance (A) at 490 nm was measured (Shimadzu UV-Vis 1800 spectrophotometer). The results were expressed in % DM compared to standard curve of glucose known concentrations.

Crude fiber content

Fiber content of cladodes was determined using the method 962.09 (AOAC, 2000). Fir this a sequential acid-base digestion method; 1 g of dried cladode powder (previously dried at 95 °C for 3 h) was boiled with 150 mL of 1.25% sulfuric acid for 30 min to remove soluble carbohydrates. The residue was then filtered, washed with hot distilled water, and boiled again with 150 mL of 1.25% KOH solution for another 30 min to remove proteins. Following a series of hot and cold-water washes and acetone rinses, the residue was dried at 105 °C to a constant weight (W1). Subsequently, the dried residue, containing both fibers and minerals, was incinerated at 550 °C for 3 h and the residue (containing minerals) was weighed (W2). Finally, the crude fiber content was calculated using the formula ($W_{Crude\ fiber}$= W1-W2) and expressed in mg/100 g of DM.

Ascorbic acid content

The content of the cladodes on ascorbic acid was determined using the method of Sarkar (1994). Briefly, to 10 mL of juice obtained by molting the cladodes, 10 mL of 20% (w/v) trichloroacetic acid were added, after homogenization, the solution was filtrated. To 10 mL of the filtrate, 1 mL of 9 M sulfuric acid and 5 mL of 0.1 M potassium dichromate were added. A control was prepared using 10 mL distilled water rather than the juice. The solutions were heated at 80 °C in water bath for 20 min, then cooled and the absorbance (A) at 582 nm (Shimadzu UV-Vis 1800 spectrophotometer) was measured. The results were expressed in mg of ascorbic acid compared to standard curve of 0.1 to 1 g/L ascorbic acid in mg for 100 g of fresh matter (FM).

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Total polyphenols content

The total polyphenols (TP) of fresh samples of the cladodes (5 g) were determined after Soxhlet extraction in 250 mL of methanol/acetone (30/70) solution for 180 min at 80 °C. To 0.2 mL of the diluted solution, 1 mL of the Folin-Ciocalteu reagent was added and the volume completed with 4.25% (w/v) sodium carbonate (Na_2CO_3) solution until 20 mL, then the solution was put in water bath at 70 °C/20 min, and the absorbance, at 760 nm, was measured (Shimadzu UV-Vis 1800 spectrophotometer) after 30 min. A blank was prepared in the same conditions. The results were expressed in mg gallic acid equivalent (GAE)/100 g FM compared to a standard curve (Singleton *et al*., 1999). All the analyses were repeated at least three times.

Prebiotic effect of the cladodes powder

Bacterial strains and culture conditions

Lactiplantibacillus plantarum F2 was isolated from fresh figs, identified using 16S rDNA sequencing and already characterized on its probiotic features (Barache *et al*., 2020*a, b*). The strain was cultivated in de Man, Rogosa and Sharpe (MRS, Condalab, Madrid, Spain) broth at 37 °C/24 h and surface streaked on MRS agar (Condalab, Madrid, Spain) with an incubation of 48 h at 37 °C. *Escherichia coli* PIUC and *Staphylococcus aureus* KB06 (from the Applied Microbiology laboratory bacterial pathogens collection, Bejaia University, Bejaia, Algeria) were included as target strains in the antibacterial and anti-biofilm activities tests. The strains were cultivated in Nutrient broth (NB, Pasteur Institute, Algiers, Algeria) or Nutrient agar (NA, Pasteur Institute, Algiers, Algeria) at 37 °C/24 h. For the biofilm assays, Tryptic soy broth (TSB, Biokar Diagnostics, France) and agar (TSA, Biokar Diagnostics, France) were used.

Preparation of the culture media

For the cladode's fibers extraction, the green extraction protocol of Cheikh Rohou *et al.* (2018) was used with some modifications. Unless water, MRS or TSB broths were directly used for fibers recovery, and the extraction was carried out under static conditions for 1h. After the preparation of MRS and TSB broths without glucose, *Opuntia ficus-indica* cladodes powder (P1 or P2) was added at different concentrations (1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5%; *w/v*) in each medium. The obtained mixture was then put in a water bath at 60 °C/1 h, for maceration, then centrifuged (8000 *g*/20 min) at 4 °C (HETTIC, ROTINA 380, Germany) and the supernatant was either autoclaved at 120 °C/20 min or filter-sterilized through 0.45 µm pore-size Acrodisc® Syringe filters (Pall Gelman Laboratory, USA). The former supernatant was designated as AS and the later as FS.

Strengthening of the strain potential by the cladodes powder

For the evaluation of the enhancement effect of the cladodes powder on the already observed *L. plantarum* F2 activities, the culture of the strain was performed in either non supplemented standard MRS broth (20 g/L glucose), referred as MRS, and used as a control, or MRS broth, without sugar, supplemented with different concentrations of the powder (1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5.0%; *w/v*), designated as MRS-Cx (x=1-9), each number correspond to one of the following concentrations (1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5.0%; *w/v*) respectively. Similarly, to assess the potential of biofilm formation, the culture of the strain was performed in either non supplemented standard TSB (2.5 g/L), referred as TSB, and used as a control, or TSB, without sugar supplemented with different concentrations of the powder (1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5.0%; *w/v*),

designated as TSB-Cx (x=1-9), each number correspond to one of the tested concentrations (1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5.0%; *w/v*) respectively.

Determination of the cladodes powder fermentability by the probiotic strain

The fermentability, and by extension prebiotic potential, of the cladodes powder was evaluated by measuring the growth of the probiotic strain *L. plantarum* F2 in the presence of different concentrations of the tested powder using a previously described method (Mueller *et al.*, 2016). In brief, the bacterial strain was incubated in MRS medium (10 g of peptone from casein, 8 g of meat extract, 4 g of yeast extract, 1 g di-potassium hydrogen phosphate, 2 g Tween 80, 2 g of di-ammonium-hydrogen citrate, 5 g of sodium acetate, 0.2 g of magnesium sulfate, and 0.04 g manganese sulfate, 20 g glucose per liter) overnight. All ingredients were obtained from Sigma-Aldrich (Steinheim, Germany). After washing the bacteria using phosphate buffered saline (PBS, 0.01 M, pH 7.2) three times, the cells were resuspended in standard MRS-broth or MRS-Cx prepared as described above. Bacteria were incubated in a final volume of 10 mL and a starting load of 10^3 CFU mL^{-1} at 37 °C for 48 h. After the incubation period, the colonies were enumerated on MRS agar and cells number was determined. All samples were tested three independent times. The average cells number was determined and compared to the cell number obtained in the presence of positive control glucose. To calculate the increase in the growth rate in the presence of the powder, the cell number of the positive control (MRS) was subtracted from the cell number of the tested sample (MRS-Cx) or *vice-versa*.

Effect on the probiotic strain auto-aggregation

The effect on the aggregation ability of the probiotic strain was assessed according to the method reported by Kos *et al.* (2003). Briefly, 18-h cultures of *L. plantarum* in MRS-Cx and MRS broths were centrifuged (8000 *g*/10 min, 4 °C) and the recovered pellets were washed three times with sterile 0.01 M PBS (pH= 7.2). Then, the pellets were resuspended in the same buffer to reach a cell concentration of 10^8 CFU/mL. After that, bacterial suspensions were vortexed, and auto-aggregation was determined after standing for 2 and 4 h at 37 °C. At the end of each incubation period, 1 mL from the upper part of the suspensions was gently aspirated and its absorbance was read at 600 nm (Specord® spectrophotometer, Shimadzu, Germany).

The auto-aggregation percentage was calculated using the following formula:

$$Auto\text{-}aggregation\ (\%) = (1-[At/A0]) \times 100 \quad [1]$$

At: Absorbance at time t = 2 or 4 h and A0: Absorbance at t = 0 h.

Effect of the cladodes powder on the antibacterial activity

The spot on- lawn method was used to assess the antibacterial activity of the probiotic strain as described previously (Barache *et al*., 2020b). For this, 10 µL of the bacterial culture in MRS or MRS-Cx were tested. *E. coli* and *S. aureus* at 10^6 CFU/mL were seeded in Muller Hinton (MH, Condalab, Madrid, Spain) agar. Sterile MRS and MRS-Cx broths filtrates were used as negative controls. Each essay was performed in three independent experiments using independently grown cultures.

Effect of the cladodes powder on biofilm formation potential

Lactiplantibacillu plantarum was tested for its biofilm formation potential using the Tissue Culture Plate (TCP) method as previously described by Bouhrour *et al.* (2023) with few modifications. A volume of 100 µL of a fresh bacterial suspension (18 h, 10^8 CFU/mL) in TSB was added to the wells of sterile flat-bottom 96-wells polystyrene microtiter plates already filled with either 100 µL TSB or TSB-Cx, and then incubated at 37 °C. After two hours of incubation, the bacterial suspensions were carefully removed and the wells were washed twice with 200 µL of sterile Tryptone-Salt (TS, saline water 9.0 g L^{-1} NaCl+0.1 g/L tryptone) solution. Then, 200 µL of either sterile TSB or TSB-Cx were added, and the plates were re-incubated for 24 h at 37 °C. Wells containing 200 µL of sterile TSB or TSB-Cx were used as negative controls. The bacterial culture was removed, and the wells were washed twice with sterile TS solution to remove the non-adherent cells. After this, 200 µL of absolute ethanol (Sigma-Aldrich, Steinheim, Germany) was added for 15 min to fix adhered cells, then the ethanol was aspirated, and the wells left to dry. After that, the fixed cells were stained with 200 µL of violet crystal 0.1 (w/v, Biochem Chemopharma, Québec, Canada) for 20 min, and then the wells were washed three times with 200 µL of sterile TS solution. Finally, the fixed stain was solubilized with 200 µL of 96% ethanol (Sigma-Aldrich, Steinheim, Germany). Biofilms biomass was quantified by reading the absorbance (A) of each well at 630 nm using a microplate reader (ELX800. BioTek, USA). Each experiment was performed three times using independently grown cultures. The classification of the strains on their adhesion capacity was done according to Stepanovic *et al*. (2000) as presented in Table 1.

Table 1. Interpretation of absorbencies in the TCP method (Stepanovic *et al.*, 2000).

Non adherent strain	Weakly adherent strain	Moderately adherent strain	Strongly adherent strain
A≤Ac (control)	Ac<A≤2xAc	2xAc<A ≤ 4xAc	4xAc<A

A: Absorbance, Ac: Absorbance of the control (sterile TSB or TSB-Cx)

Effect of the cladodes powder on pathogens biofilm formation inhibition

The effect of cladodes powder on the anti-biofilm potential of *L. plantarum* was assessed against two pathogenic strains *E. coli* and *S. aureus*. For this, the wells of a flat bottom 96- well microtiter plate were filled with 100 µL of sterile TSB and 50 µL of *L. plantarum* cell free supernatant (CFS recovered from TSB or TSB-Cx and designated as CFS-TSB or CFS-TSB-Cx respectively), prepared as previously described. Then, 50 µL of fresh pathogen bacterial culture (18 h, 10^7 CFU/mL) were added. After two hours at 37 °C, the bacterial suspensions were removed, and the wells were washed twice with 200 µL of sterile TS solution. Then, sterile TSB was added, and the plates were re-incubated for 24 h at 37 °C. Wells filled with only CFS-TS, CFS-TSB-Cx or pathogen bacterial suspension was used as negative and positive controls respectively. For the evaluation of the anti-biofilm activity of the culture supernatant, the bacterial suspension was removed, and the wells were washed twice with 200 µL of sterile TS solution. The adhered cells were fixed with 200 µL of absolute ethanol for 15 min, then the ethanol was aspirated, and the wells were left until dryness. Afterwards, the fixed cells were stained with 200 µL of violet crystal 0.1% (w/v) for 20 min and then washed with 200 µL of sterile TS solution three times. Finally, the fixed stain was solubilized with 200 µL of 96% ethanol. The effects of the CFS on biofilm formation were assessed by reading the absorbance (A) of each well at 630 nm using a microplate reader (ELX800. BioTek, USA).

The % of biofilms inhibition was calculated as follows:

$$\% \text{ Biofilm inhibition} = \frac{[(A_{630\text{ nm positive control}} - A_{630\text{ nm experimental}}) * 100]}{A_{630\text{ nm positive control}}} \quad [2]$$

Each experiment was performed three times using independently grown cultures.

Effect of the cladodes powder on the antioxidant potential

The antioxidant potential was determined using the DPPH radical scavenging capacity as previously described by Barache *et al.* (2024). For this, 100 µL of CFS, recovered either from MRS or MRS-Cx (designated CFS-MRS or CFS-MRS-Cx respectively), was mixed with 1 mL of a methanolic solution of DPPH (0.04 mg/mL). The mixture was kept in the dark for 30 min and then the absorbance was measured at 515 nm (Specord® spectrophotometer, Shimadzou, Germany). This assay was performed in three independent experiments using independently grown cultures. The radical scavenging capacity (RSC) was calculated using the following formula:

DPPH RSC (%) = 1-(A0/As) × 100 [3]

A0: The absorbance of the DPPH radical solution without CFS
As: The absorbance of the DPPH radical and CFS mixture

Effect on the in vitro cholesterol lowering

Determination of cholesterol lowering ability was conducted as follows. A solution of 3.0 g/L cholesterol was prepared by dissolving cholesterol > 99% purity (Sigma-Aldrich, Steinheim, Germany) in 99% ethanol and tween 80 (Sigma-Aldrich, Steinheim, Germany), mixed in 3:1 ratio according to Ziarno (2008). A volume of 1 mL of the prepared solution was added to 100 mL of MRS or MRS-Cx broth supplemented with 0.3% of porcine bile (Sigma-Aldrich, Steinheim, Germany). The medium was then inoculated with 10^8 CFU/mL of probiotic strain and incubated for 24 h at 37 °C. After that, the cultures were centrifuged (8000×*g*/10 min, 4 °C) and the CFS (CFS-MRS or CFS-MRS-Cx) was used for cholesterol quantification. Non-inoculated MRS or MRS-Cx broth was included as control. Assimilation of cholesterol was calculated as loss of its concentration in MRS broth CFS after 24- h culture at 37 °C. Cholesterol concentration in CFS was assayed with the enzymatic diagnostic test Cholesterol RTU® 142 (BioMérieux, France). Absorbance at 505 nm was measured with a spectrophotometer (Specord® 143 Schimadzu, Germany). This assay was performed in three independent experiments using independently grown cultures. Percentage value of cholesterol assimilation was calculated using the following formula:

Cholesterol assimilation (%) = [1-(*A*-*B*)/*C*] x 100 [4]

where: *A*= [cholesterol] in 24- h *L. plantarum* FB3 culture (MRS or MRS-Cx broth+ cholesterol solution), *B*= [cholesterol] in 24-h *L. plantarum* FB3 culture (MRS or MRS-Cx broth without cholesterol solution), *C*= [cholesterol] in sterile MRS or MRS-Cx broth+ cholesterol solution.

Statistical analysis

Differences between samples were calculated using one-way ANOVA and the post hoc Tukey test ($p < 0.05$) XL-STAT (version 2009, Addinsoft, Paris, France), and data were expressed as a mean ± standard error calculated from at least three independent experiments.

Results and discussion

Chemical analysis of the cladodes

The chemical composition of the *Opuntia ficus-indica* cladodes is summarized in Table 2.

Table 2. Chemical composition (%, *w/w*) of young (03-06 months old) and old (>1-year-old) *Opuntia ficus-indica* cladodes.

Component	03-06 months	Cladode age > 1 year
Water	36.45 g/100 g (FM)	45.45 g/100 g (FM)
Minerals	25 g/100 g (DM)	28 g/100 g (DM)
Ca	7.66 mg/100g (DM)	8.72 mg/100g (DM)
Mg	0.61 g/100g (DM)	0.68 g/100g (DM)
K	10.51 mg/100g (DM)	11.43 mg/100g (DM)
Total sugars	9.60% (DM)	6.67% (DM)
Total crude fibers	9.54 mg/100 g (DM)	10.57 mg/100 g (DM)
Ascorbic acid	11.32 mg/100g (FM)	12.08 mg/100g (FM)
Total Polyphenols	6.59 mg GAE/100g (FM)	7.52 mg GAE/100g (FM)

DM: Dry Matter; FM: Fresh Matter

The composition of the cladodes includes water, polysaccharides, fibers, proteins, vitamins, fatty acids, sterols, polyphenols, and minerals. It varies according to different factors, such as plant age, growing season, soil factors, and growth area (Caminiti *et al*., 2024). According to Rodrigues *et al*. (2023), *O. ficus indica* cladodes have a very high nutritional value in vitamins, mainly ascorbic acid, and minerals, such as magnesium (Mg), calcium (Ca), and potassium (K), as well as being rich in antioxidants like phenolic compounds and flavonoids.

Moisture and minerals

The content in water in the cladodes was 36.45% (*w/w*) in the young individuals (3-6 months old) and 45.45% (*w/w*) in the aged ones (>1 year-old). In the literature, the values reported varied between 80 and 95% (Silva *et al.*, 2021; Hernandez-Becerra *et al.*, 2022). The low water content of the analyzed cladodes could be explained by the dry period of the sampling (summer, 45 °C). The minerals content was 25% (young cladodes) and 28% (aged cladodes), levels higher than the values of 18.57 ± 7.57% DM and 21.35 ± 1.86% DM, reported by Guevara-Figueroa *et al.* (2010) and Hernández-Urbiola *et al*. (2010). According to Feugang *et al.* (2006), cladodes are rich in minerals (Ca, Na, K, Mg, Fe, Cu, Zn, Mn, and Ni), they could reach a threshold of 23% DM, with dominance of calcium (5.64 mg/100g DM) and potassium and (2.35 mg/100g DM) (Feugang *et al.*, 2006; Ayadi *et al.*, 2009). In our analysis, the values of Ca content are 7.66 mg/100g (young cladodes) and 8.72 mg/100g (aged cladodes). For the K, the contents were 10.51 mg/100g (young cladodes) and 11.43 mg/100g (aged cladodes). El Mostafa *et al.* (2014) have reported large interval of Ca and K values in *O. ficus indica* cladodes that varied from 5.64 to 17.95 mg/100 g and 2.35 to 55.20 mg/100 g respectively. For magnesium, the

results showed values of 0.61 mg/100g (young cladodes) and 0.68 mg/100g (aged cladodes), which are very lower than the value of 8.8 mg/100 g reported by El Mostafa *et al.* (2014). Higher levels of 7518 ± 162 mg/100 g of Ca, 1684 ± 68 mg/100 g of K and 1380 ± 137 mg/100 g of Mg were reported by Missaoui *et al*. (2020). According to Ayadi *et al*. (2009) and Contreras-Padilla *et al.* (2012), oscillation of the minerals level in the cladodes could be linked to the growth conditions such as salinity and the physicochemical characteristics of the soil, the maturity stage, and the presence of thorns.

Sugars and fiber content

The sugars content varied between the young and the aged cladodes. It was 9.60% (DM) in the young cladodes and 6.67% (DM) in the aged ones. It was reported that sugars are the macromolecules the most abundant in the cladodes of *Opuntia* (64-80.9% of DM) and their level is proportional to the agronomic and environmental variations as well as the age of cladodes; the young ones are the richest in sugars (Ginestra *et al.*, 2009; Guevara-Figueroa *et al.,* 2010). Glucose and galacturonic acid are the main sugars in *Opuntia* cladodes (Belhadj Slimen *et al.*, 2016). Regarding the fiber, the values ranged between 9.54% (DM) in the young cladodes and 10.57% (DM) in the aged ones. According to the literature, the cactus cladodes contain high quantities of fiber (until 51.6% DM), including mucilage, pectin, lignin, cellulose, and hemicellulose (Ayadi *et al*., 2009; Guevara-Figueroa *et al.*, 2010). Soluble fibers, which dissolve in an aqueous solution, include pectins, mucilage, and certain types of hemicelluloses (Peña-Valdivia *et al.*, 2012). Insoluble fibers include cellulose and hemicelluloses (Weickert and Pfeiffer, 2008; Ramirez-Tobias *et al*., 2012) and lignin (Slavin, 2008). In the study of Rojas-Molina *et al.* (2015), soluble and insoluble fiber contents in the cladodes powder samples were 2.53 ± 0.90 and 43.44 ± 1.69%, respectively, whereas Hernandez-Urbiola *et al.* (2010) reported values of 8.0 and 52.0%, respectively in cladodes of ≈ 3 months of age. A higher content of insoluble fiber compared to soluble ones is due to the formation of polyphenolic polymer lignin, because of lignification, a natural maturation step in cladodes. On the contrary, young *Opuntia* cladodes lack lignin (Peña-Valdivia *et al.*, 2006).

Ascorbic acid and polyphenols content

The ascorbic acid and polyphenols varied slightly between young and aged cladodes. The values ranged between 11.32-12.08 mg/100g (FM) and 6.59-7.52 mg/100g (FM) respectively. It was reported that *O. ficus indica* cladodes are rich in vitamins (vitamin C, α-tocopherol, β-carotene, xanthin, and betanin) (Feugang *et al.*, 2006). Values of 7-22 mg/100 g and 12.7 mg/100 g of ascorbic acid were reported by El Mostafa *et al.* (2014) and Hernandez-Becerra *et al.* (2022) respectively. One class of compounds contained in cladodes and responsible for beneficial activities are polyphenols, that level is influenced by the plant age (Figueroa-Pérez *et al.,* 2018). The main identified polyphenols in the cladodes are quercetin, naringin, ferulic acid, piscidic acid, eucomic acid, quinic acid, malic acid, p-coumaric acid 3-O-glucoside, tricin, hydroxyl octadecadienoic acid, eicosanoic acid, rutin, kaempferol-rutinoside, and narcissin, among others (Silva *et al*., 2021; Caminiti *et al*., 2024). El Mostafa *et al.* (2014) reported the richness of *Opuntia* cladodes on polyphenols and Ayadi *et al*. (2009) mentioned a value of 825.81 ± 24.13 mg/100 g of DM.

Prebiotic effect of the cladodes powder

After 48 h of incubation at 37 °C, the results showed (Figure 3) that the probiotic strain *L. plantarum* F2 demonstrated its ability to ferment the powder sugars and expressed a similar or significantly better (p<0.05) growth in MRS-Cx broth (0.0, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5.0%). In the presence

of P1, significantly higher growth rates (p<0.05) were seen compared to glucose with an increased growth rate ranged from 0.38 log at the concentration of 1.0 to 1.06 log at 5.0% in the autoclaved supernatant (AS). Best growth enhancement was observed in the MRS-Cx filtered supernatant (FS), with an increase in the growth rate from 1.04 log (1.0% P1) to 2.09 log (5.0% P1), differences were highly significant (p<0.01) compared to the control (glucose). These results suggest that maceration of the cladodes powder P1 has efficiently stimulated the growth of the probiotic strain, which can have important implications for its use in food formulations or food supplements such as sugar substitute and prebiotic. Similarly, the results obtained with the powder P2 indicated the fermentability of the powder sugars, but no significant effect (p>0.05) in the probiotic strain growth was noticed in the culture medium prepared by maceration and autoclaving compared to the control (glucose). However, in the filtered medium, a significant increase (p<0.05) in the growth was recorded in the concentration range (2.0 to 5.0% of P2) with an increase of 0.34 to 1.09 log respectively. These results indicate that the preparation by maceration of the medium enriched with P2 can enhance the strain growth, but this is dependent on the concentration and the sterilization method used.

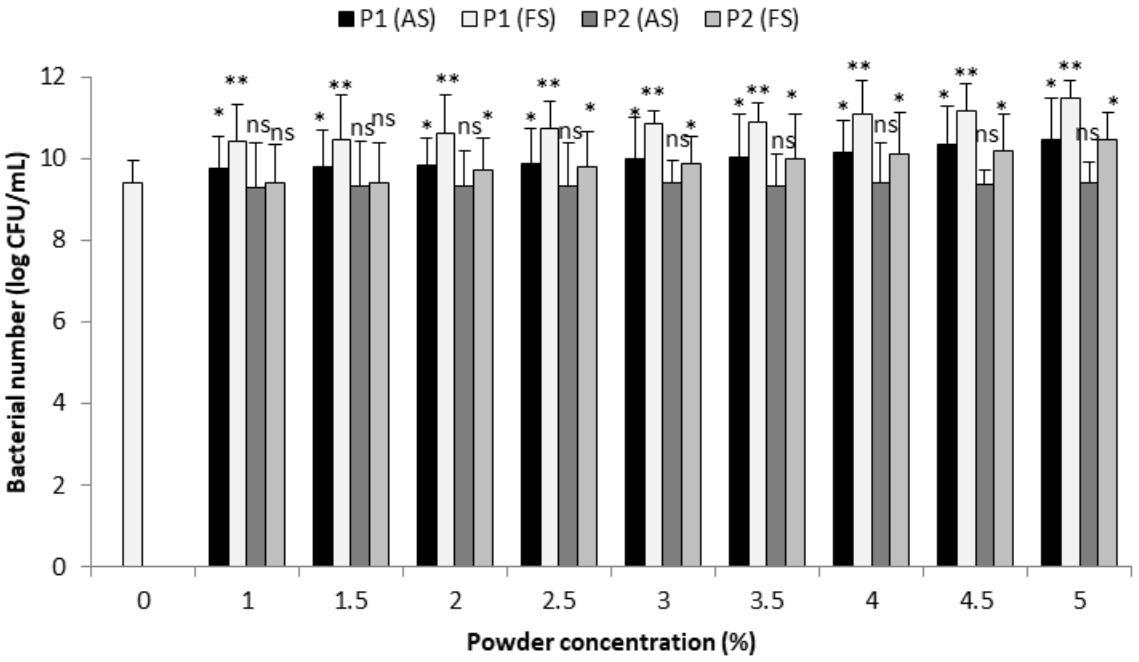

Figure 3. Growth of *L. plantarum* F2 in MRS (0% powder) and MRS-Cx in the presence of different concentrations of the powder P1 or P2 of *Opuntia ficus-indica* cladodes (0.0-5.0% w/v). White bar: negative control (MRS without addition of powder); AS: Autoclaved MRS-Cx supernatant; FS: Filtered MRS-Cx supernatant. n=3 independent experiments each in triplicate. Bars with asterisk indicate a significantly higher growth ($^{*}p$<0.05, $^{**}p$<0.01, Mann Whitney u test) in the presence of the indicated cladodes powder concentration than the control. ns: not significantly different than the control.

In this study, a significant growth of the probiotic strain was observed in the MRS supplemented with the cladodes powder, which testified on its richness on fermentable fibers. It's interesting to mention that the pH of the MRS (initially pH=6.3) was adjusted to pH=5.3 to concord with that of the supplemented MRS-Cx, which dropped instantly to pH=5.3 after addition of the powder. As is known

lactobacilli grow easily in acidic media and pH 5.4 is the ideal pH for lactobacilli selection. The obtained results indicate that the extraction method used (60 °C/1 h) was efficient in extraction of sufficient quantity of soluble fibers to allow *Lactobacillus* strain growth. It's well known that *Opuntia ficus-indica* cladodes present an interesting prebiotic effect linked mainly to their composition on complex polysaccharides (pectins, mucilage, hemicelluloses and cellulose) (Weickert and Pfeiffer, 2008; Peña-Valdivia *et al*., 2012). According to Weickert and Pfeiffer (2008), colonic fermentation of naturally available high fiber foods can be mainly attributed to soluble dietary fibers. Cladodes are rich in mucilage, a complex organic substance, mainly composed of supramolecular assemblies of neutral and acidic polysaccharides, with the possibility to include low molecular compounds such as oligosaccharides. These oligosaccharides are usually neglected in complex structures suggesting an interesting prebiotic potential (Cruz-Rubio et *al.*, 2021). Our results are better than that reported by Cruz-Rubio *et al.* (2020), who observed a lesser ability of *Lactobacillus* and *Bifidobacterium* strains to use mucilage of *Opuntia* as substrate to grow compared to glucose. This difference could be attributed to the strain used in our study, which belongs to *L. plantarum*, a species that presents a strong carbohydrate utilization capability, which contributes to a broad adaptability in various environments with different carbohydrates. This species could not only use cellobiose, mannose, D-ribose, and L-fucose, but also ferment prebiotics fructo-oligosaccharides (FOS) and galacto-oligosaccharide (GOS) (Cui *et al*., 2021). Furthermore, in the present study, the procedure used (maceration at 60 °C/1 h) has probably improved the fibers content of the MRS broth (MRS without glucose) and fermentation by the *Lactobacillus* strain may allow enrichment of the medium on nutritive elements. Akanni *et al.* (2015) have already highlighted that cultivation of yeasts *Candida utilis* and *Kluyveromyces marxianus* have improved the total protein content of an enzymatic cladode hydrolysate. In the study of Caminiti *et al.* (2024), extraction at 60 °C/3 h of insoluble fibers was more efficient when using cladodes collected in August than that collected in February. According to them, a temperature difference of 27 °C between the cladodes collected in winter (9 °C) and summer (36 °C) could explain the difference in insoluble fibers concentrations present in the two samples, since fibers are used by the plant as a water tank in summer. So, maceration in aqueous phase could be promoted as efficient method to allow enrichment of liquid products on the *Opuntia ficus-indica* cladodes bioactive compounds. Maceration is an extraction process in which a solid component is kept immersed in a liquid solvent for a variable time, depending on the type of solvent used and the compounds to be extracted. Experimentally, the vegetable ingredient of interest must, first, be chopped into small pieces to increase the contact with the solvent and allow it to penetrate the innermost cells. At the same time, the sample should not be pulverized in order not to lose its volatile active ingredients and to make filtration possible at the end of the process. Once the ideal size is reached, the solid component is immersed in the solvent for a variable time. At the end of the maceration time, the macerate is gently filtered and used, while the insoluble residue is eliminated (Gori *et al.,* 2021). Fermentation could also enhance the bio-disponibility of soluble fibers. Regarding this, Tan *et al*. (2024) have used *L. plantarum* C11- fermentation-assisted extraction method to extract fibers from radish. They found that this method seems the best way to extract white radish soluble dietary fibers compared to other methods. On the other hand, some of the phenolic compounds act as prebiotics and stimulate the growth of species and thus modulate the composition of human gut microbiota (Makarewicz *et al*., 2021).

Effect on the probiotic cells auto-aggregation

The auto-aggregation rates, obtained after 4 h of incubation, were better for all the tested concentrations (30-90% of auto-aggregation) compared to the control (26.0 ± 0.6% of auto-

aggregation), with 5.0% (w/v) as the concentration allowing the highest auto-aggregation rates (75 ± 0.3 and 90 ± 0.1% for P1 [AS] and P1 [FS]; 56 ± 0.5 and 79 ± 0.3% for P2 [AS] and P2 [FS] respectively). Although the highest percentages of auto-aggregation were established at 5.0%, aggregation values increased in a concentration-dependent manner (Figure 4). Our results demonstrated that the auto-aggregation of the probiotic strain was enhanced when grown in the presence of the cladodes powder. The highest levels were registered in the filtered supernatant of either P1 or P2, significant differences (p< 0.05) were recorded compared to the autoclaved supernatant (Figure 4).

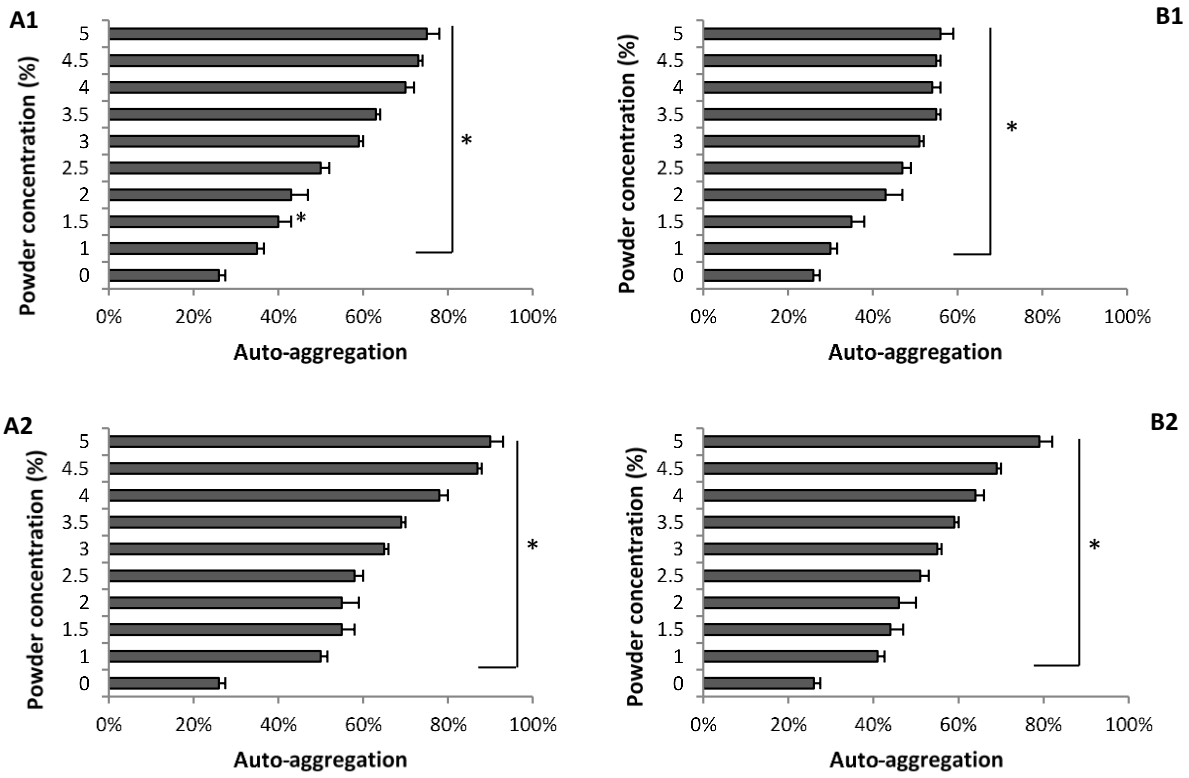

Figure 4. Percentage of auto-aggregation monitored after 4 h of incubation. Each assay was conducted by triplicate. Means and standard errors are shown. Columns with an asterisk are significantly different (p< 0.05) from the control, using one-way ANOVA with Tukey test for pairwise comparisons.

Auto-aggregation is an important feature of probiotic strains; it promotes adhesion and colonization of the human gut. The studied strain *L. plantarum* F2 was already demonstrated able to produce large quantity of exopolysaccharides (EPS) (Barache *et al.*, 2020a). The EPS structure can act as a capsule bound to the cell surface, allowing bacterial protection and contribute to aggregation and adhesion. Fermentation of fibers could stimulate EPS production by probiotic strain. Furthermore, Preska Steinberg *et al.* (2019) have demonstrated that polymers such dietary fibers (pectins) could induce aggregation of bacteria in the intestine. So, the increase in the probiotic strain auto-aggregation, registered in the presence of cladodes powder, in dose dependent manner, could be due to the action

of the dietary fibers contained in the cladodes. This finding is very interesting, suggesting the benefit association of cladodes dietary fibers and probiotics in diet.

Effect of the cladodes powder on the antibacterial activity

To evaluate the antibacterial activity of *L. plantarum* against *S. aureus* and *E. coli*, the spots on lawn test were used. The obtained results showed that the *L. plantarum* strain cultivated in MRS-Cx (in the presence of P1 or P2) had better antibacterial activity than that recorded in MRS (Figure 5). Interestingly, total inhibition of *E. coli* (10^6 CFU/mL) was observed with all the tested concentrations of P1 and P2. The initial activity of *L. plantarum* grown in standard MRS was 4.3 ± 0.3 cm. It must be mentioned that the sterile P1 and P2 macerates had an antibacterial activity against the two pathogens (≤1.5 ± 0.1 cm in diameter) in the concentrations range (2.5-5%, AS and FS) and this activity increased in function of the powder concentration from 0.5 ± 0.07 to 1.5 ± 0.1 cm. No activity was recorded with the negative control used (MRS broth) against the two strains. Concerning the *L. plantarum* activity against *S. aureus*, an inhibition zone of 3.65 ± 0.03 cm in diameter was observed when the probiotic strain was grown in standard MRS, whereas in the presence of P1, the results were more interesting with an increase in inhibition zones diameters to 5.5 ± 0.15 to 5.8 ± 0.11 cm in the autoclaved supernatant (AS). Similarly, in the filtered supernatant (FS) inhibition zones of 6.68 ± 0.01 to 7.8 ± 0.07 cm were also observed. These observations suggest that the growth in the presence of the powder macerate improve the antibacterial efficacy of *L. plantarum*, this could be linked to growth stimulation as shown above or high production of the antibacterial substances or synthesis of other substances not produced in the presence of glucose. On the contrary, in the broth enriched with P2, weak increase or decrease in the inhibition zones diameters was observed; values varied in the range of 3.35 ± 0.1 cm to 4.35 ± 0.11 cm from the higher to the lower concentration in AS. Same trend was observed in FS with weak increases (3.85 ± 0.02 to 4.90 ± 0.04 cm). These results indicate that P2 had a variable effect on the antibacterial activity of the probiotic strain depending on concentration and sterilization method.

Opuntia ficus- indica cladodes are rich in dietary fibers that could exert a prebiotic effect on the used probiotic strain *L. plantarum* F2. Morrison and Preston (2015) reported the stimulating role of natural dietary fibers on beneficial bacteria growth and the production of short chain fatty acids (SCFA) allowing the regulation of intestinal transit and the lipids metabolism. As mentioned by these authors, the fermentation of fibers plays a key role in the protection against the gastro-intestinal diseases by allowing an increase of the SCFA production, alike butyrate and propionate, and a decrease of the generated amount of ammonia.

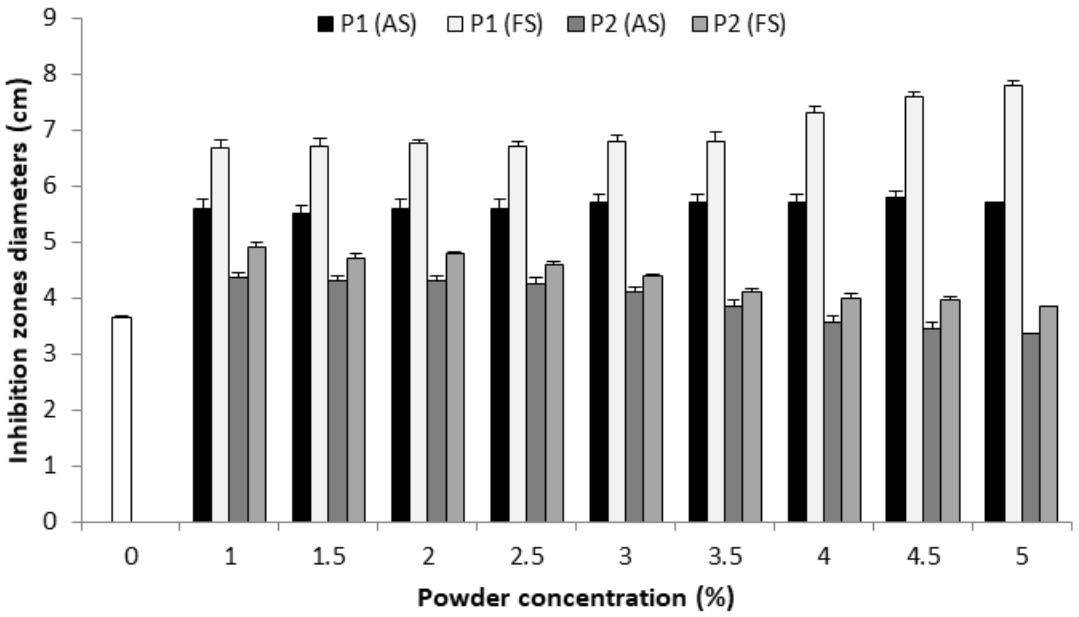

Figure 5. Antibacterial activity of *L. plantarum* (spots on- lawn test) against *S. aureus* in the presence of different concentrations of the *Opuntia ficus-indica* cladodes powders (P1 or P2) in cm. White bar: negative control (standard MRS); AS: Autoclaved MRS-Cx supernatant; FS: Filtered MRS-Cx supernatant. n=3 independent experiments each in triplicate.

Effect of the cladodes powder on biofilm formation potential

The effect of *Opuntia ficus-indica* cladodes powder (P1 and P2) on the adhesion potential of the probiotic strain *L. plantarum* F2 was tested by the semi quantitative method of adhesion on polystyrene microplates. The results are indicated in Table 3.

Table 3. Adhesion of *Lactiplantibacillus plantarum* F2 on polystyrene microplates in the presence of different concentrations of the powders P1 and P2 of *Opuntia ficus- indica*.

Medium tested	None adhesion	Weak adhesion (%)	Moderate adhesion (%)	Strong adhesion (%)
Without powder	-	TSB+ 0 P	-	-
With maceration and filtration	-	-	TSB +5.0 P1 TSB +1.0 P2 TSB +5.0 P2	TSB +1.0 P1
With maceration and autoclaving	-	-	TSB +1.0 P1 TSB +5.0 P1 TSB +1.0 P2 TSB +5.0 P2	-

-: None medium

The results showed (Table 3) that P1 and P2 improved significantly the adhesion of *L. plantarum* and its biofilm formation potential. However, *L. plantarum* showed a gradual improvement in its adhesion from weak adhesion (TSB+ 0% P), to moderate with strong adhesion in filtered supernatant of TSB +1.0% P1. The results of the adhesion test in TSB enriched with *Opuntia ficus-indica* cladodes

powders showed an improvement in the adhesion of the probiotic strain compared to its adhesion in the standard TSB. These observations are correlated to those obtained for the aggregation potential of the probiotic strain in the presence of the cladodes powder. The adhesion potential favors the colonization and increases the bacterial persistence in the host mucosa and unable the colonization by the pathogens (Monteagudo-Mera *et al.*, 2019).

Effect of the cladodes powder on pathogens biofilm formation inhibition

The results of the adhesion of *E. coli* and *S. aureus* and the anti-adhesive effect of *L. plantarum* at the different concentrations of P1 and P2 against the two target strains are shown in the Figure 6. The results showed (Figure 6) that the probiotic strain had a weak anti-adhesive effect (16.04% inhibition) against *E. coli* in standard TSB. In the presence of P1 or P2, more significant adhesion inhibition was registered, with 77.54 and 89.30 in AS+1% P2 respectively. Inhibition rates of 39.57 in AS+1% P1 and FS+5% P2 were recorded.

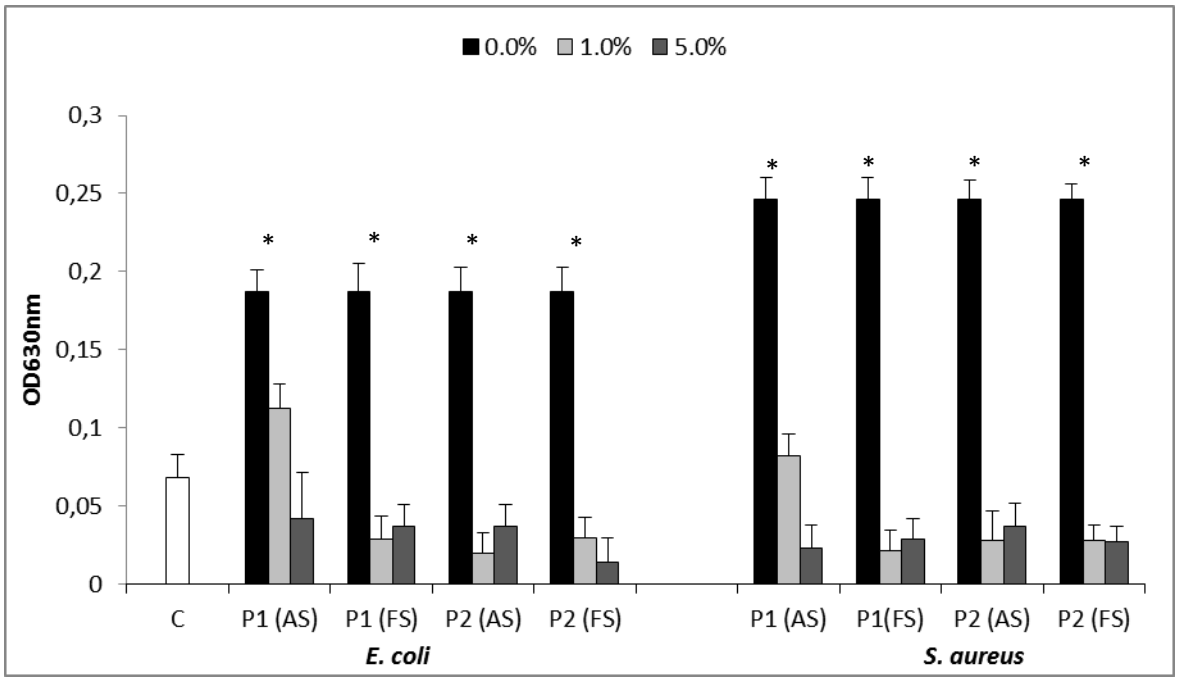

Figure 6. Anti-adhesive effect of *L. plantarum* supernatant on *E. coli* and *S. aureus* on polystyrene microplates grown in TSB enriched by different concentrations of *Opuntia ficus-indica* cladodes powder P1 or P2. AS: Autoclaved supernatant, FS: Filtered supernatant. C: Negative control (sterile TSB or TSB-Cx broth). Columns with asterisk indicate significant differences (p<0.05) compared to positive control (standard TSB). TSB-Cx broth indicates TSB supplemented with the two concentrations (1.0 and 5.0%) of P1or P2.

Similarly, the results of the inhibitory effect of the probiotic strain on *S. aureus* in the standard TSB was 30.89%. In the presence of P1, a great increase in the adhesion inhibition effect was registered varying from 66.66 with AS+1% P1 and 91.05 with FS+1% P1. These results indicate that TSB enriched with the different concentrations of P1 and P2 had a positive effect on the anti-adhesive effect of the probiotic *L. plantarum*. The CFS from the probiotic strain was able to prevent the pathogens adhesion and subsequently the biofilm formation of *E. coli* and *S. aureus* based on the results obtained with the semi quantitative TCP method (Figure 6). Complete inhibition was registered at 5.0% of

powder. The antimicrobial activity of *Lactobacillus* strains is attributed to the production of diverse antimicrobial substances, such as organic acids, mainly lactic acids, which can control pathogens proliferation. They can also inhibit their biofilm formation capacity or interfere with their quorum signaling pathways (Barache *et al*., 2020a). This *Lactobacillus* strain of vegetal source produces 15.88 ± 0.22 g L^{-1} lactic acid (Barache *et al.*, 2020b).

Effect of the cladodes powder on the antioxidant potential

In order to study the antioxidant potential of lactobacilli stains, the DPPH radical scavenging assay was used. Remarkably, the culture supernatants of *L. plantarum* exhibited high DPPH scavenging activities, varying from 56.15 ± 0.34 to 65.54 ± 0.52% (Table 4). This strain showed a DPPH scavenging percentage of 51.30 ± 1.45% in standard MRS.

Table 4. Antioxidant potential of *L. plantarum* F2 in function of cladodes powder concentration.

[Powder] %	P1 (AS) %	P1 (FS) %	P2 (AS) %	P2 (FS) %
0.0		51.30 ± 1.45^{a}		
1.0	51.45 ± 0.55^{a}	54.07 ± 0.78^{b}	50.27 ± 1.86^{a}	51.33 ± 0.06^{a}
1.5	53.14 ± 1.43^{b}	54.31 ± 1.23^{b}	51.45 ± 0.46^{a}	54.25 ± 1.54^{b}
2.0	54.04 ± 0.64^{b}	56.56 ± 0.82^{c}	51.67 ± 0.879^{a}	56.25 ± 0.86^{c}
2.5	56.08 ± 0.18^{c}	57.34 ± 1.63^{c}	53.34 ± 1.65^{b}	59.25 ± 1.06^{d}
3.0	57.04 ± 0.67^{c}	57.43 ± 1.03^{c}	53.55 ± 1.09^{b}	61.20 ± 0.43^{d}
3.5	56.13 ± 1.38^{c}	58.09 ± 1.22^{c}	54.18 ± 0.34^{b}	61.56 ± 1.36^{d}
4.0	56.45 ± 0.49^{c}	60.16 ± 1.23^{d}	54.88 ± 0.06^{b}	63.70 ± 1.06^{e}
4.5	60.31 ± 1.90^{d}	63.87 ± 0.56^{e}	56.45 ± 1.43^{c}	63.86 ± 1.06^{e}
5.0	60.16 ± 0.53^{d}	63.45 ± 0.33^{e}	56.15 ± 1.31^{c}	66.25 ± 0.56^{f}

Results are expressed as mean ± standard deviation n = 3, means with different lowercase letters were significantly different (p< 0.05) based on Tukey's test.

As mentioned by Mishra *et al.* (2015), the DPPH assay is generally used *in vitro* to determine the scavenging activity, and is one of the most sensitive, common, and reliable methods. Many studies have revealed that antioxidant activity of *Lactobacillus* strains might be linked to their production of cell-surface compounds, e.g., lipoteichoic acid and EPS, and to antioxidant enzymes, such as superoxide dismutase, NADH-oxidase, and NADH-peroxide, and heterologous non-haem catalase (Li *et al.*, 2012; Yang *et al*., 2019). Antioxidant properties, by decreasing the level of oxidants such as reactive oxygen species (ROS), are among the most interesting attributes of probiotics (Mu *et al.*, 2019). When comparing the antioxidant potential, a difference was observed between the culture media; this could be related to the reactionary environment generated by each cladodes powder concentration, but it could also be due to the enhancement of the potential of the probiotic strain, especially linked to its antioxidant mechanisms and the produced metabolites. Indeed, many mechanisms could be used by probiotics to reduce oxidative damage: antioxidant enzymes system (*e.g*., superoxide dismutase and catalase), metal ions chelation, antioxidant metabolites (*e.g*., glutathione, butyrate, and folate), and positive regulation of host antioxidant activities (Wang *et al*., 2017). Otherwise, *O. ficus-indica* cladodes contain vitamins (C and E), antioxidants and various flavonoids, particularly quercetin 3-methyl ether, a highly efficient radical scavenger (El Mostafa *et al*., 2014). The polyphenols present in this plant, such as flavonoids, stilbenes, phenolic acids, lignin and suberin, exert an antioxidant activity by inhibiting the oxidation process via interactions with the sugars, lipids, and proteins (Jakobek, 2015). The flavonoids neutralize the free radicals, chelate the pro-

oxidant metallic ions, inhibit the pro-oxidant enzymes, and induce the expression of antioxidant enzymes. The stilbenes scavenge the free radicals, inhibit the oxidation of lipids, and regulate the expression of the genes implicated in the oxidative response. The phenolic acids neutralize the free radicals, inhibit the pro-oxidant enzymes, and stimulate the production of endogen antioxidant enzymes. Although less studied, the lignin and suberin showed antioxidant activities by scavenging the free radicals and stabilizing the cellular membranes (Belhadj Slimen *et al*., 2016). The betalaines, thanks to their hydroxyle groups, imino and tetrahydropyridine, inhibit the lipids peroxidation and the disintegration of the heme (Taira *et al*., 2015). Dok-Go *et al.* (2003) showed that quercetine, dihydroquercetine and quercetine-3-methyl-ether, present in the fruit and the cladodes, are able to reduce the deleterious effects caused by the hydrogen peroxide and the xanthine oxidase in the rat cortical cells. Moreover, *O. ficus-indica* polyphenols, together with other components (saponins, sterols, lignans, and some vitamins), play important protective roles in some metabolic diseases, such as metabolic syndrome, hypercholesterolemia, and obesity, as well as hypertension, asthma, and rheumatic pain (Petruk *et al*., 2017). In this study, significant antioxidant effect was noticed in the cladodes powder. This may show the efficacy of the dehydration (in the sun) and extraction (maceration in water) methods used. Moreover, better antioxidant activity was observed in the filtered supernatants rather than the autoclaved ones and in the aged cladodes than the young ones. Dehydration is a preservation process used for many years, which is based on reducing the water content by reducing its water activity (aw). An advantage of dehydrated products is that additives are not usually used to preserve; therefore, they are considered natural foods with the consequent advantages. The lower the temperatures used in the drying process the lesser is the degradation of the products (Rodríguez-Garcia *et al.*, 2007). Different results were obtained in this work compared to the literature, where young cladodes were reported expressing better antioxidant activity than the old ones (Caminiti *et al.*, 2024). Indeed, insoluble fibers significantly reduced the accumulation of ROS and stronger antioxidant power was observed in cladodes collected in summer than that in winter and in younger than aged cladodes, even though no significant differences were registered in polyphenols contents. This could be due to the fermentation of the macerate by the probiotic strain, which enhances the biodisponibility of the phenolic compounds and thus the antioxidant potential as demonstrated by Ait Chait *et al*. (2019) for carob pods. On the other hand, dietary polyphenols can undergo biotransformation during the activity of bacterial enzymes, leading to the formation of different phenolic derivatives characterized by small and low molecular weight as well as a modified biological activity (Makarewicz *et al*., 2021).

Effect on the in vitro cholesterol lowering

Remarkably, the strain was able to reduce more significantly ($p < 0.05$) the cholesterol concentration in the macerate of the cladodes powder (56.2 ± 1.16 to 90.04 ± 0.33%) compared to the control (53.5 ± 1.13%). The highest cholesterol removal rates were obtained in the filtered macerate of 5.0% P1 (90.04 ± 0.33%) and P2 (84.25 ± 1.56%). The reduction was proportional to the powder concentration (Table 5).

Table 5. Cholesterol lowering ability of *L. plantarum* F2 in function of cladodes powder concentration.

[Powder] %	P1 (AS) %	P1 (FS) %	P2 (AS) %	P2 (FS) %
0.0		53.5 ± 1.13		
1.0	60.13 ± 1.15	64.67 ± 0.78	56.20 ± 1.16	58.33 ± 0.86
1.5	61.41 ± 1.33	66.08 ± 1.23	58.35 ± 1. 26	60.25 ± 1.34
2.0	65.54 ± 0.89	71.13 ± 0.82	61.28 ± 1.09	65.25 ± 1.06
2.5	71.78 ± 1.48	78.43 ± 1.63	68.55 ± 1.17	70.25 ± 0.86
3.0	76.01 ± 0.56	80.75 ± 1.03	70.75 ± 1.89	75.25 ± 1.03
3.5	79.43 ± 0.78	85.68 ± 1.22	73.48 ± 0.55	78.25 ± 1.06
4.0	81.38 ± 0.97	86.45 ± 1.23	76.87 ± 0.86	80.25 ± 1.36
4.5	85.46 ± 0.49	89.09 ± 0.56	79.85 ± 1.43	83.25 ± 1.46
5.0	86.75 ± 1.43	90.04 ± 0.33	80.45 ± 1.56	84.25 ± 1.56

Results are expressed as mean ± standard deviation $n = 3$, means with different lowercase letters were significantly different ($p < 0.05$) based on Tukey's test.

The ability of lactobacilli to reduce cholesterol level *in vitro* has been shown for many strains such as *L. acidophilus*, *L. delbrueckii* subsp. *bulgaricus*, *L. casei*, *L. gasseri*, *L. amylovorus* (Ziarno, 2008), *L. pentosus* (Bendali *et al.*, 2017), *L. plantarum* and *L. paracasei* (Barache *et al.*, 2020a). According to Kumar *et al.* (2012), probiotics could use many mechanisms to lower cholesterol levels, adsorption of cholesterol to bacterial walls and its incorporation into cellular membrane are the most documented mechanisms used by lactobacilli to reduce cholesterol levels. *In vivo*, the possession of a bile salt - hydrolase (BSH) is suggested as the main mechanism underlying the hypocholesterolemic potential of probiotic lactobacilli strains (Barache *et al*., 2020a). On the other hand, and according to Galati *et al*. (2003), daily oral administration of lyophilized cladodes to hyperlipidemic rats was associated with a reduction in plasma cholesterol, LDL, and triglyceride levels, without affecting HDL levels and they linked this effect to the soluble fiber contained in the dry cladodes. They also suggested the involvement of flavonoids and ascorbic acid in this reduction. Similarly, Weickert and Pfeiffer (2008) and Caminiti *et al*. (2024) reported that *O. ficus-indica* can prevent hypercholesterolemia and consumption of soluble dietary fibers reduces total and LDL cholesterol levels. Moran-Ramos *et al.* (2017) tested the effect of powdered cladodes in diet of obese rats, and a decrease of total cholesterol serum levels was observed. Furthermore, Padilla-Camberos *et al.* (2015) have demonstrated an inhibition of the pancreatic lipase; a key enzyme implicated in fats absorption and suggested its contribution in the hypocholesterolemic effect. So, a synergistic effect could explain the enhancing hypocholesterolemic potential of *L. plantarum* cultured in the presence of *O. ficus-indica* macerate.

Conclusions

In this study, a macerate of *Opuntia ficus-indica* cladodes powder in MRS or TSB broths was used as a bacterial culture medium to underline the potential of this cactus as a source of soluble fibers and bioactive compounds and their influence on the bacterial growth and probiotic features of the probiotic strain *Lactiplantibacillus plantarum* F2. In the light of our findings, the strain can use the macerate of *Opuntia ficus-indica* as substrate and the fibers act as prebiotics to enhance growth and beneficial effects of the probiotic strain. The results obtained in this paper lead to four important conclusions:

(1) Water maceration of *O. ficus-indica* cladodes powder generates fermentable fibers;
(2) Cladodes soluble fibers can exert prebiotic effects;

(3) *O. ficus-indica* macerate enhances the probiotic strain features (growth, aggregation, and adhesion);
(4) Synergistic effect was observed between the probiotic strain and *O. ficus-indica* cladodes powder (increase of the antibacterial, anti-biofilm, antioxidant and hypocholesterolemic potentials).

So, it seems that integration of *Opuntia ficus-indica* cladodes powder in probiotic formulations can represent an innovative approach for the reinforcement of the efficacy and viability of probiotic strains. This synergy could allow numerous opportunities for the development of functional products with nutritional and therapeutic benefits and contributing to the improvement of the global health.

ETHICS STATEMENT

Not applicable

COMPETING INTERESTS

The authors declare that they have no competing interests.

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