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Prebiotic Influence on Probiotic Bacteria: Growth and Acidification Dynamics

HEENA CHOUDHARY, DIPJYOTI CHAKRABORTY, ATUL SHARMA[2], JATINDRA KUMAR PRADHAN[1] AND RAJ SINGH*[2]

Department of Bio-sciences and Biotechnology, Banasthali Vidyapith, Newai, Tonk-304 022 (Rajasthan), India
**(e-mail: dr.rajsingh09@gmail.com; Mobile: 98979 90346)*

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ABSTRACT

In this study, the *in vitro* effects of five commercially available prebiotics: fructo oligosaccharides (FOS), inulin (INU), galacto-oligosaccharides (GOS), xylo-oligosaccharides (XOS) and lactulose (LAC) were compared for the growth and acidifying activity against five probiotic bacterial strains: *Bacillus subtilis*, *Bacillus amyloliquefaciens*, *Bacillus cereus*, *Bacillus pumilus* and *Brevibacterium* spp. Three concentrations of the prebiotics: 0.5, 1 and 2% were evaluated. The kind and concentration of prebiotics were found to have a significant effect on the probiotic strains' proliferation and acidifying action. In general, growth and acidifying activities enhanced as prebiotic contents raised. Notably, *Bacillus* strains showed consistent growth enhancement, with XOS and FOS performing particularly well. However, *Brevibacterium* species exhibited significant species-specific responses, with GOS and XOS showing the most beneficial effects on growth. Additionally, the study found that higher concentrations of prebiotics promoted greater acidifying activity, with *B. subtilis* displaying the highest acidification in response to XOS. These findings highlight the importance of selecting appropriate prebiotics for each probiotic strain in the development of functional foods. The results emphasize the role of prebiotics in enhancing probiotic performance and suggest that their efficacy varies based on the strain and type of prebiotic.

Key words: Prebiotics, probiotic bacteria, growth, acidifying activity, *Bacillus* strains, *Brevibacterium* sp.

INTRODUCTION

The health benefits of probiotics and prebiotics have recently caught the interest of both food makers and consumers. Many new functional foods and supplements that contain both prebiotics and probiotics have been developed (Bisht *et al*., 2024). Synbiotics, which combine these two components, are designed to work synergistically. As long as pathogenic and dangerous bacteria do not take over, the human gastrointestinal tract (GIT) supports the host's regular physiological functions as a dynamic micro-ecosystem. It has been proposed that regularly adding probiotics, prebiotics, or synbiotics to the diet can help maintain a healthy balance of the microbiota in the gastrointestinal tract (GIT) (Rehman *et al*., 2020). Probiotics, which come from the Greek term meaning "for life," are live bacteria that actively support health by balancing the gut microbiota when taken in enough proportions. Improved intestinal motility is one of the many health advantages linked to probiotic microorganisms, enhanced natural resistance to intestinal infections, prevention of diarrhoea, reduced serum cholesterol, alleviation of lactose intolerance, better nutrient absorption, protein pre-digestion and the preservation of mucosal integrity (Kuerman *et al*., 2020). Probiotics have traditionally been included in fermented foods such as yogurt. More recently, they have been incorporated into beverages and sold as supplements in various forms, including freeze-dried preparations, pills, and capsules. The concept of prebiotics in nutrition has become both increasingly intriguing and complex. The non-digestible food component known as a prebiotic enhances the host's health by favourably encouraging the proliferation and/or activity of certain bacteria in the colon (Nunpan *et al*., 2019). According to the 2004 update, prebiotics are defined as

[1]Department of Zoology, Government Autonomous College, Phulbani-762 001 (Odisha), India.
[2]Department of Bio-Sciences and Technology, Maharishi Markandeshwar (Deemed to be University), Mullana, Ambala-133 207 (Haryana), India.

nutrients that have undergone selective fermentation, resulting in specific alterations in the composition and/or activity of the gastrointestinal microbiota, which enhances the health and well-being of the host. Any food component that can enter the colon has the capacity to function as a prebiotic (Edwards *et al*., 2020). Nonetheless, a few standards permit a food item to be categorized as a prebiotic. Among them are:

(a) It must not be absorbed or hydrolysed in the upper gastrointestinal tract.
(b) Selective fermentation in the colon, driven by potentially beneficial bacteria.
(c) A shift towards a more healthful makeup of the colonic microbiota.
(d) Ideally, provide outcomes that improve the health of the host.

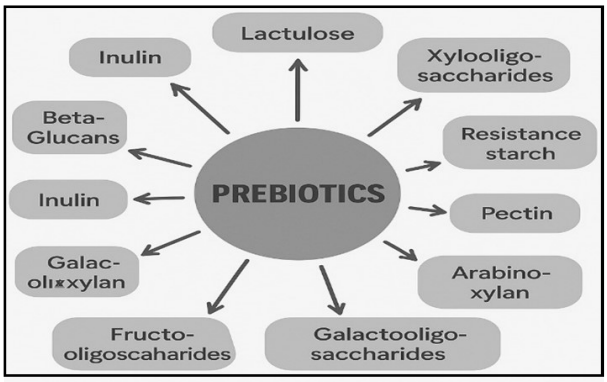

Fig. 1. The different types of prebiotics.

Using a range of technologies, several prebiotic friendly carbohydrates have been widely identified as oligo- and polysaccharides from a variety of natural sources and are already commercially accessible (Fig. 1). There are a number of prebiotic oligosaccharides in the market, including gentio-oligosaccharides, mannan-oligosaccharides, galacto-oligosaccharides, soybean oligosaccharides, xylo-oligosaccharides, lactulose and fructo-oligosaccharides (Peng *et al*., 2020). Although some bacteria are promoted as probiotics, most descriptions are associated with the genus *Lactobacillus*. The *Bacillus* species are an interesting class of probiotic bacteria that hasn't got much attention (Fara *et al*., 2020). *Bacillus* species are constantly being researched and have shown great probiotic potential because of their long shelf life, viability at room temperature and under refrigeration, and capacity to survive in foods that require harsh processing conditions, such as high temperature and pressure (Sirbu *et al*., 2022). As probiotic supplements, *Bacillus* often requires a lower effective dose than lactic acid bacteria. However, despite its advantages, prospective *Bacillus* strains must undergo safety testing. *Bacillus cereus*, *Bacillus anthracis* and other species that are generally thought to be benign, such *Bacillus subtilis*, have been linked to food-borne diseases and the manufacture of toxins (Maraz *et al*., 2022). Among the high G+C actinomycetes, the genus *Brevibacterium* represents a distinct line of descent. Although, *Brevibacterium* species can grow on a range of media, EYGA medium allows for the clearest observation of their morphology and staining reactions. When strains are cultivated on complex media, they typically exhibit a clear rod-coccus cycle (Iqbal *et al*., 2023).

Common laboratory media such as yeast extract, malt extract agar, yeast extract, peptone agar, blood agar, nutrient agar and tryptone soy agar are ideal for *Brevibacterium* species growth (Dong *et al*., 2024). When NaCl is added to the media, *Brevibacterium* species tolerate it and may occasionally exhibit increased activity. *Brevibacterium* species are chemoorganotrophic, aerobic, catalase-positive actinomycetes with an oxidative metabolism. Limited research has been conducted to investigate the patterns of antibiotic susceptibility in *Brevibacterium* species (Jr *et al*., 2021). Currently, the most efficient method for differentiating *Brevibacterium* species from other actinomycetes, especially those with a rod-coccus development cycle, is 16S rRNA gene sequencing.

Previous research has shown that prebiotics significantly enhance the growth and activity of probiotics (Kaewarsar *et al*., 2023). The influence of prebiotics on the development and function of probiotic bacteria has been explored in several *in vitro* and *in vivo* studies (Edwards *et al*., 2020). However, most studies have focused on a limited number of prebiotics or saccharides with prebiotic properties, examining their effects on the human gut microbiota or a small selection of probiotics (Kaewarsar *et al*., 2023). However, the nutritional and physiological benefits of oligofructose and inulin have received the greatest attention from academics (Fara *et al*., 2020). Probiotic development and viability are

significantly influenced by prebiotics, making the selection of appropriate prebiotic ingredients crucial when developing functional foods that combine both probiotics and prebiotics. The variations between bacterial strains and the development and activity of putative probiotic bacteria in media treated with various prebiotics, seem to be amenable to *in vitro* examination (Delgado-Fernandez *et al*., 2019). The finger millet ML365 variety was used to isolate four possible probiotic bacteria from *Bacillus* and one from *Brevibacterium* sp. in order to assess the impact of commercially available prebiotics on the development and acidifying properties of these bacteria. The purpose of this study was to examine these effects *in vitro* (Abouloifa *et al*., 2020).

MATERIALS AND METHODS

Previous studies investigated the isolation and characterization of probiotic bacteria from fermented finger millet flour (*Eleusine coracana*, variety ML365) to evaluate their potential in functional food development. Researchers isolated 25 bacterial strains, from which 12 gram-positive, catalase-negative rod-shaped isolates were selected based on morphological and biochemical screening (Choudhary *et al*., 2025). Among these, five isolates (EC01-EC05) were further characterized due to their promising probiotic properties. The selected isolates exhibited typical lactic acid bacteria (LAB) traits, including carbohydrate fermentation and resilience under simulated gastrointestinal conditions. These isolates demonstrated strong tolerance to acidic pH (2-3) bile salts (0.3-0.5%), phenol (0.2-0.4%) and high NaCl concentrations (up to 9%).

Earlier, findings also reported antibiotic resistance profiles, with all five isolates showing resistance to ciprofloxacin, vancomycin and tetracycline, while remaining sensitive to erythromycin and ampicillin. Functional probiotic characteristics such as hydrogen peroxide and bacteriocin production, auto-aggregation, co-aggregation with *E. coli* and surface hydrophobicity were also assessed. Notably, EC01 showed the highest antimicrobial activity and adhesion potential. Molecular identification through 16S rRNA sequencing confirmed EC01 as *B. subtilis*, EC02 as *B. amyloliquefaciens*, EC03 as *Brevibacterium* sp., EC04 as *B. cereus* and EC05 as *B. pumilus*. Compatibility assays indicated that EC01, EC04 and EC05 could be combined in probiotic consortia without antagonistic interactions. These isolated probiotics were used to check the activity of the prebiotics (Choudhary *et al*., 2025).

Commercial preparations of galacto-oligosaccharide (GOS; Farmland Bio, India), fructo-oligosaccharide (FOS; Farmland Bio, India), inulin (INU; Farmland Bio, India), xylo-oligosaccharide (XOS; Farmland Bio, India) and lactulose (LAC; Sigma) were utilized as prebiotics. Three distinct concentrations of the prebiotics: 0.5, 1 and 2% (w/v) were examined. Membrane filters with a particle size of 0.45 µm (Millipore) were used to filter sterilizing stock solutions containing 10% prebiotic compounds in distilled water.

For the *Lactobacillus* and *Brevibacterium* spp. cultures, the basal growth medium used was MRS broth without carbohydrates. To get final prebiotic concentrations of 0.5, 1 or 2%, sterile prebiotic solutions were added to the basic MRS broth. The prebiotic supplemented baseline growth medium was supplemented with a 1% activated bacterial culture. The positive control was the basal growth medium supplemented with 2% glucose, while the negative control was the basal growth medium by itself. The pour plate method using MRS agar was employed to determine the initial viable cell counts of the inoculated growth medium. Following inoculation, plates were incubated in both the anaerobic and aerobic conditions for 24 h at 37°C. Following prebiotic incubation, the pour plate method using MRS agar was employed to determine the number of viable cells in the culture fluid. To evaluate the effect of prebiotics on the growth performance of probiotic bacteria, the viable cell count after the incubation time was compared to the initial viable cell counts in the baseline medium. A pH meter was used to measure the acidifying activity of the colonies. The study contained three duplicates of each prebiotic (Edwards *et al*., 2020).

To assess the viability of *B. subtilis* cells under prebiotic treatment, fluorescence microscopy was performed using the live/dead BacLight Bacterial Viability Kit (Molecular Probes, Invitrogen). This staining technique utilizes SYTO 9, which penetrated all bacterial cells and fluoresces green, and propidium iodide (PI),

which only entered cells with compromised membranes and fluoresces red (Ruan *et al*., 2020).

Bacterial cultures were grown in MRS broth supplemented with 1% fructo-oligosaccharide (FOS), while control cultures were maintained in prebiotic-free basal MRS medium. After 24 h of incubation at 37°C under aerobic conditions, 1 ml of each culture was harvested by centrifugation at 8000 rpm for 10 min, washed twice with sterile phosphate-buffered saline (PBS) and re-suspended in 1 ml PBS.

A 1:1 mixture of SYTO 9 and PI stains was added to the bacterial suspension and incubated in the dark at room temperature for 15 min. A 10 µl aliquot was mounted on a clean glass slide and covered with a cover slip. Fluorescence images were captured using a fluorescence microscope (Olympus BX53) equipped with appropriate FITC (green) and TRITC (red) filter sets. All images were obtained at 1000× magnification under oil immersion. Image acquisition and analysis were performed using CellSens Standard software (Searns *et al*., 2019).

The data were described using the proper range, which was mean ± standard deviation (±SD). The student t-test was used to compare the quantitative variables between the study groups, and the paired t-test was used to compare the variables within the study groups. Multiple comparison of means was statistically analysed using both one-way and two-way ANOVA. The statistical software GraphPad Prism (Version 9.2.0) for Microsoft Windows and SPSS (Statistical Package for the Social Science) version 21 (IBM Corp., Armonk, N. Y., USA) were used for all statistical computations.

RESULTS AND DISCUSSION

The effects of prebiotic substances on the growth and acidifying characteristics of probiotic bacterial strains are displayed in Table 1 and Fig. 12. All data presented in this table are from three biological replicates (mean±). The findings showed that the probiotic strains growth performance was impacted by the kind and quantity of prebiotics added to the baseline medium. The effects of prebiotic type and concentration on the growth of *Brevibacterium* sp. were statistically significant (P<0.05). After incubation, the negative and

Table 1. The impact of prebiotic compounds on the investigated probiotic bacterial strains growth and acidifying activity

Prebiotics/ Concentration (%)	*Bacillus subtilis*		*Bacillus amyloliquefaciens*		*Bacillus pumilus*		*Bacillus cereus*		*Brevibacterium* sp.	
	Increase in the VCN log CFU/Ml	pH after incubation	Increase in the VCN log CFU/Ml	pH after incubation	Increase in the VCN log CFU/Ml	pH after incubation	Increase in the VCN log CFU/Ml	pH after incubation	Increase in the VCN log CFU/Ml	pH after incubation
Negative control[b]	0.04±0.05	2.57±0.35	0.10±0.00	2.00±0.17	0.10±0.17	2.43±0.21	0.20±0.10	2.40±0.40	0.13±0.06	2.00±0.17
Positive control[b]	0.60±0.78	1.70±0.46	0.70±0.10	1.40±0.10	0.93±0.06	1.93±0.21	0.83±0.06	1.83±0.06	0.70±0.10	1.43±0.10
FOS 0.5	0.43±0.49	1.93±0.06	1.83±0.06	0.80±0.10	0.83±0.06	2.27±0.15	0.83±0.06	1.67±0.08	1.80±0.00	0.83±0.12
1	0.83±0.06	1.97±0.12	1.87±0.06	0.80±0.10	0.80±0.10	2.13±0.06	0.80±0.10	1.93±0.32	1.70±0.10	0.77±0.06
2	0.97±0.15	1.87±0.12	1.73±0.12	0.93±0.15	0.97±0.06	2.03±0.06	0.97±0.06	1.83±0.23	1.73±0.12	0.93±0.15
INU 0.5	0.70±0.10	1.93±0.06	0.80±0.10	2.40±0.50	0.63±0.06	2.47±0.51	0.63±0.06	2.10±0.35	0.90±0.20	2.33±0.40
1	0.60±0.10	1.77±0.06	0.80±0.10	2.17±0.25	0.77±0.15	2.37±0.59	0.77±0.15	2.00±0.35	0.80±0.10	2.17±0.25
2	0.77±0.06	1.73±0.06	0.87±0.06	1.93±0.29	0.73±0.15	2.30±0.70	0.73±0.15	1.90±0.35	0.87±0.06	1.93±0.29
GOS 0.5	0.37±0.06	2.17±0.06	0.73±0.15	2.17±0.32	0.70±0.10	2.00±0.26	0.70±0.10	1.93±0.15	0.70±0.10	2.07±0.25
1	0.57±0.06	1.93±0.15	0.87±0.12	1.97±0.42	0.97±0.21	1.83±0.06	0.97±0.21	1.83±0.06	0.87±0.12	1.90±0.36
2	0.67±0.06	1.77±0.31	0.67±0.06	1.90±0.35	1.03±0.12	1.90±0.20	1.03±0.12	1.93±0.06	0.70±0.00	2.03±0.29
XOS 0.5	0.33±0.06	2.37±0.38	0.83±0.06	2.27±0.47	0.83±0.06	2.43±0.06	0.83±0.06	1.90±0.17	0.80±0.10	2.30±0.44
1	0.20±0.10	2.33±0.32	0.83±0.06	2.17±0.46	0.77±0.06	2.33±0.06	0.77±0.06	2.00±0.10	0.90±0.10	2.10±0.35
2	0.50±0.10	2.17±0.38	0.70±0.10	2.10±0.20	0.67±0.06	2.30±0.10	0.67±0.06	1.83±0.15	0.70±0.10	2.10±0.20
LAC 0.5	0.27±0.06	1.77±0.38	0.80±0.10	2.10±0.35	0.87±0.06	2.10±0.20	0.87±0.06	2.20±0.10	0.80±0.10	2.10±0.15
1	0.47±0.12	1.53±0.15	0.87±0.06	1.93±0.06	0.80±0.10	2.00±0.20	0.80±0.10	2.10±0.10	0.90±0.10	1.87±0.15
2	0.57±0.06	1.53±0.15	0.77±0.06	1.83±0.06	0.90±0.10	1.87±0.12	0.90±0.10	2.27±0.32	0.77±0.06	1.87±0.15

*VCN- Viable cell number, values are mean ± SD (n=3) and negative and positive control (basal growth medium and basal growth medium supplemented with glucose (2%).

positive controls yielded 0.13 and 0.7 log CFU / ml, respectively. There was a considerable increase in the number of viable cells after prebiotic incubation, with an increase ranging from 0.7 to 1.8 log CFU / ml.

At 1% concentration, GOS, XOS and Lac provided the optimal support for the growth of the *Brevibacterium* species. When compared to other prebiotics, the supportive growth effect of FOS and INU at 1% concentration was comparatively lower (Fig. 1). This strain's growth was significantly accelerated by FOS and INU in comparison to 2% glucose (positive control) (Fig. 2). The growth of this strain is comparatively less affected by GOS, XOS and LAC than by 2% glucose. The kind and concentration of prebiotics had a substantial ($P<0.05$) impact on *B. subtilis* growth. XOS and FOS at 0.5 and 2% were the most effective in supporting *B. subtilis* growth (Fig. 2). Nearly all prebiotics supported the growth of the probiotic bacteria at 2% concentration. However, at 0.5 and 1% concentrations, FOS, INU, GOS, XOS and Lac showed comparatively lesser growth than the positive control compared to the positive control of 2% glucose (Fig. 3).

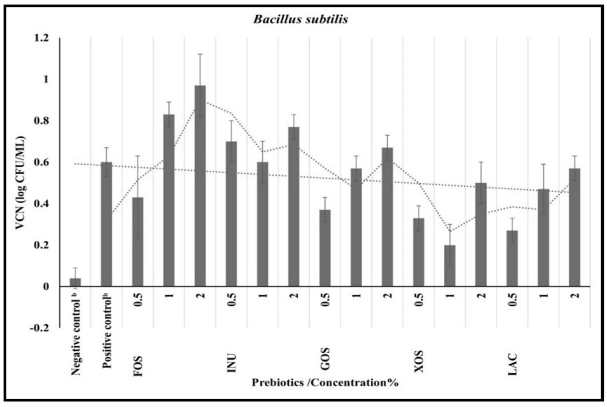

Fig. 2. The viable cell number of the *B. subtilis* at different concentrations of the prebiotics.

The growth of *B. amyloliquefaciens* was significantly influenced by both the type and concentration of prebiotics ($P<0.05$). This strain had fewer viable cells initially than after incubation with the prebiotics. The viable cell counts for the negative and positive controls were 0.1 and 0.7 CFU / ml, respectively, after incubation. The prebiotics increased the number of viable cells from 0.1 to 1.8 CFU / ml (Fig. 4). FOS had the greatest impact on the growth of *B. amyloliquefaciens* at concentrations of 0.5 and 1%. FOS at 2% exhibited a relatively

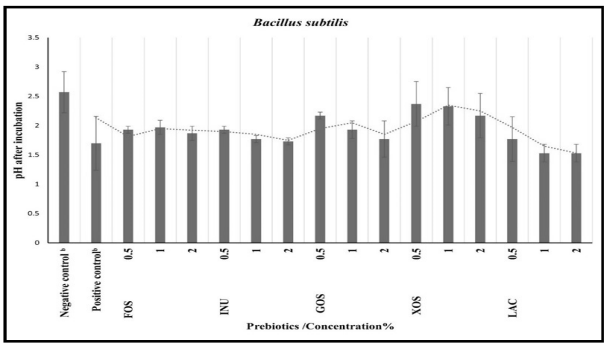

Fig. 3. The pH of the *B. subtilis* after incubation at different concentrations of the prebiotics.

moderate growth-supporting effect on this strain, while only GOS at 1% concentration showed a stronger growth-supporting effect. XOS and INU, on the other hand, also significantly increased the number of viable cells; the greatest effects were shown at concentrations of 2, 0.5 and 1%, respectively (Fig. 5).

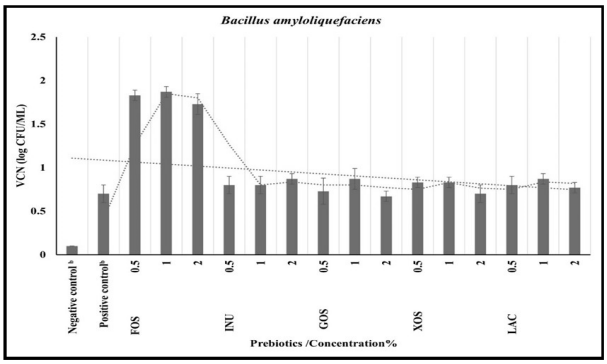

Fig. 4. The viable cell number of the *B. amyloliquefaciens* at different concentrations of the prebiotics.

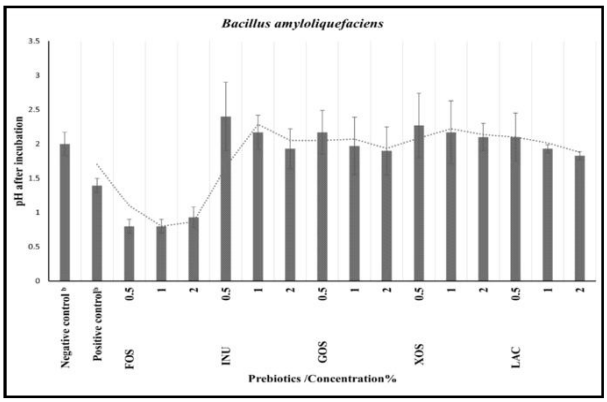

Fig. 5. The pH of the *B. amyloliquefaciens* at different concentrations of the prebiotics.

B. cereus growth was significantly impacted by both the kind and concentration of prebiotics ($P<0.05$). This strain's initial viable cell count

was between 0.15 and 0.2 CFU/ml. The number of viable cells significantly increased following prebiotic incubation (Fig. 6). At 2% concentration, XOS provided the best support for *B. cereus* growth. Compared to FOS and GOS, LAC and INU at 2% concentration demonstrated a comparatively smaller supportive growth effect on this strain (Fig. 7). GOS had the greatest impact on *B. pumilus* growth at 2% concentration. At 2% concentration, the growth effect of INU and XOS was comparatively less than that of GOS, which was the only substance that had a larger supporting effect on this strain's growth than the 2% glucose (Fig. 8). However, at 2% concentration, the supportive effects of FOS and LAC were comparable to those of 2% glucose (Fig. 9).

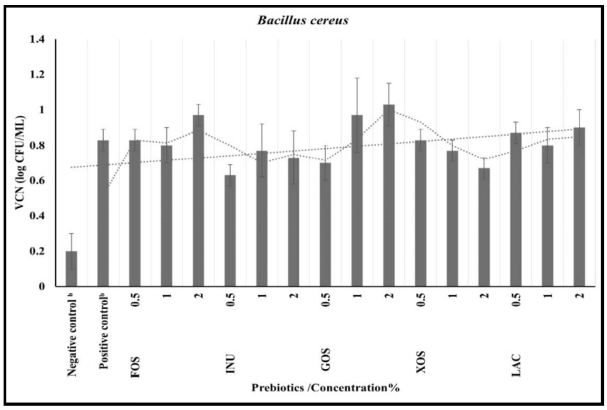

Fig. 6. The viable cell number of the *B. cereus* at different concentrations of the prebiotics.

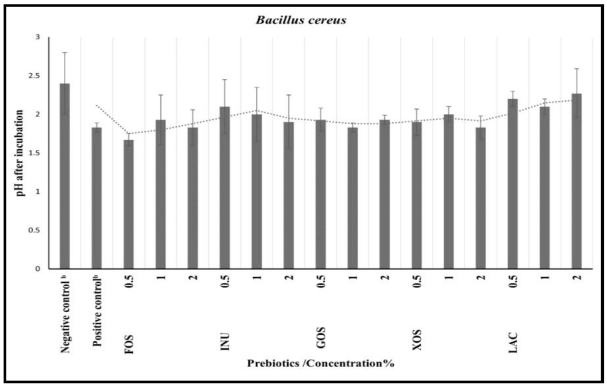

Fig. 7. The pH of the *B. cereus* at different concentrations of the prebiotics.

Most prebiotics were found to positively influence acidifying action as their concentration increased. The pH values of the prebiotic-containing culture media ranged from 0.7 to 2.5 for each type of bacterium. *B. subtilis* exhibited the highest pH value (2.3),

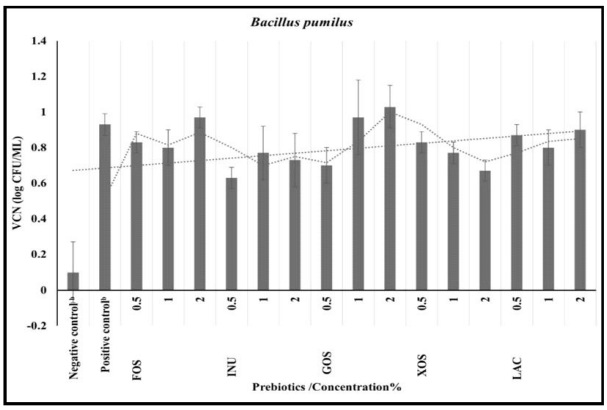

Fig. 8. The viable cell number of the *B. pumilus* at different concentrations of the prebiotics.

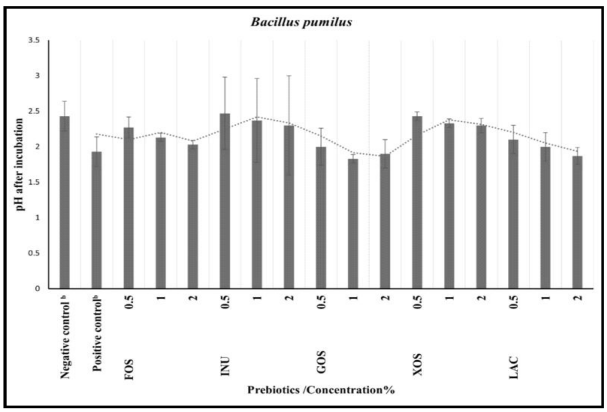

Fig. 9. The pH of the *B. pumilus* at different concentrations of the prebiotics.

surpassing the positive control, when XOS was used at 0.5 and 1% concentrations of the prebiotic. At 0.5% concentrations, INU and XOS induced the strongest acidifying activity in *B. amyloliquefaciens* and *B. pumilus*, followed by LAC, GOS and FOS. However, the 0.5% FOS concentration had a relatively minor effect on *B. cereus*. LAC and INU showed the strongest acidifying action at 0.5%, though these

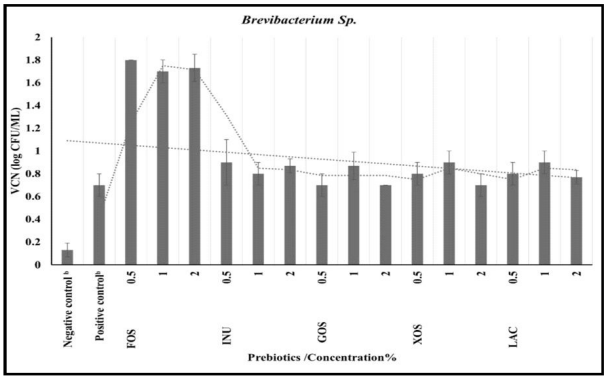

Fig. 10. The viable cell number of the *Brevibacterium* sp. at different concentrations of the prebiotics.

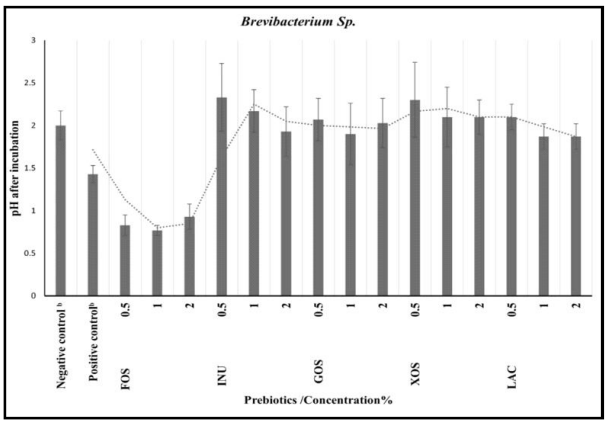

Fig. 11. The pH of the *Brevibacterium* sp. at different concentrations of the prebiotics.

prebiotics resulted in a smaller increase in the viable cell count of the strain compared to others (Fig. 10). Notably, FOS exhibited the weakest acidifying activity with *Brevibacterium* species in comparison to the other prebiotics (Fig. 11).

The study's findings showed that the kind and quantity of prebiotics had a significant impact on the probiotic bacterial strains ability to thrive and their acidifying activities (Fig. 12). This study's findings regarding the beneficial impact of prebiotics on the probiotic bacterial strain's growth performance are consistent with other studies (Markowiak and Œli¿ewska, 2017). As their concentration increased, the prebiotics typically had a positive effect on the probiotic strains' acidifying activities (Hutkins *et al*., 2025). As the probiotic strains' viable cell counts rose, comparatively greater acidifying actions were noted. The strains of the bacterial species had no effect on growth performance or acidifying activities (Bisht *et al*., 2024). Nonetheless, a significant species difference was observed within the *Brevibacterium* genus ($P<0.05$). Additionally, a number of studies showed that the strain and/or substrate of probiotic bacteria may affect their capacity to use prebiotics (Delgado-Fernández *et al*., 2020). In conclusion, the appropriate prebiotic source

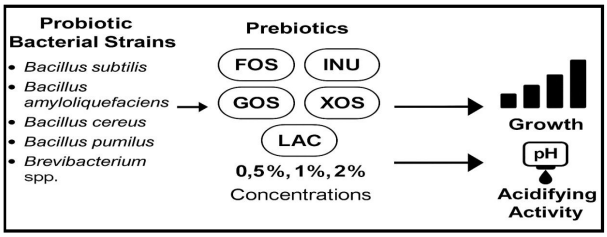

Fig. 12. Impact of prebiotics on growth and acidifying activities of probiotics.

should be selected for each probiotic bacterial strain based on its capacity to acidify, support healthy growth and endure before developing functional meals that blend prebiotics with probiotics as a synbiotics.

Fluorescence microscopy revealed a clear difference in the viability of *B. subtilis* cells when grown with and without prebiotic supplementation. In the control group (Fig. 13A), most cells exhibited faint or patchy green fluorescence, and a few cells showed compromised membrane integrity, suggesting suboptimal viability under nutrient-limited conditions. In contrast, the cells treated with 1% FOS (Fig. 13B) demonstrated a higher density of bright grey fluorescence, indicating a larger proportion of metabolically active and intact cells. There was a noticeable reduction in whitish (dead) cell signals, confirming that FOS enhanced bacterial survival and membrane integrity. These visual findings correlate strongly with the quantitative results of viable cell counts and acidifying activity observed earlier. The fluorescence images provide further confirmation that prebiotic treatment promotes cellular viability, thus supporting the synbiotic potential of combining *B. subtilis* with FOS.

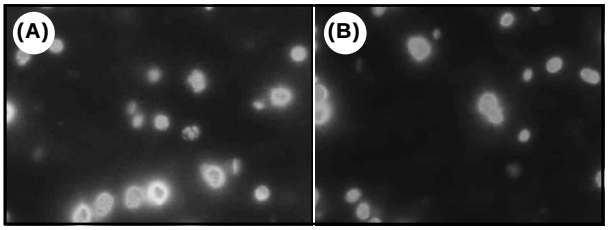

Fig. 13. Fluorescence microscopy images of *B. subtilis* viability. (A) Control without prebiotics showing limited grey fluorescence, indicating fewer viable cells and (B) Cells treated with 1% FOS showing intense grey fluorescence, representing higher viability. Stain using SYTO 9 (grey for live cells) and propidium iodide (whitish for dead cells). Images captured at 1000× magnification and scale bar = 10 µm.

CONCLUSION

The prebiotics are non-digestible substrates that stimulate growth of probiotic, playing vital roles in supporting gut microbiota balance, digestion, nutrient absorption, immune function, metabolic health and even mental well-being when combined into synbiotics.

These agents can act synergistically: prebiotics enhance probiotic survival and colonization, while the probiotics metabolize prebiotics into bioactive compounds such as short-chain fatty acids, bacteriocins and antioxidants, which yield additive or complementary health effects. The kind and quantity of prebiotics had a significant impact on the probiotics to acidify, support healthy growth and endure before developing functional meals. This study confirmed that supplementation with 1% FOS significantly improved the viability of *B. subtilis*.

Fluorescence microscopy showed enhanced grey fluorescence and reduced whitish signals, grey colours showing healthier cells. These observations aligned with the increased viable counts and acidifying activities. FOS improved membrane integrity and metabolic activity under nutrient-limited conditions. The results supported the use of *B. subtilis* with FOS as a promising synbiotic combination. Such formulations may enhance microbial stability and functional benefits in probiotic applications.

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REFERENCES

Abouloifa, H., Khodaei, N., Rokni, Y., Karboune, S., Brasca, M., D 'Hallewin, G. and Asehraou, A. (2020). The prebiotics (Fructo-oligosaccharides and Xylo-oligosaccharides) modulate the probiotic properties of *Lactiplanti bacillus* and *Levilacto bacillus* strains isolated from traditional fermented olive. *World J. Micro. Biotech*. **12**: 1-12. *doi:10.1007/s11274-020-02961-9*.

Bisht, D., Pal, D. and Shrestha, R. (2024). Introduction to probiotics, prebiotics and synbiotics. *Probiotics*, CRC Press, **28**, eBook ISBN-9781003452249.

Choudhary, H., Chakraborty, D., Sharma, A., Pradhan, J. K., Behl, R. K., Sharma, P., Anjali Kanwal and Singh, R. (2025). Isolation and characterization of probiotic bacteria from *Eleusine coracana* (Finger millet) -ML365 fermented flour. *Annl. Biol.* **41**: 39-45.

Delgado-Fernández, P., Corzo, N., Olano, A., Hernández-Hernández, O. and Moreno, F. J. (2019). Effect of selected prebiotics on the growth of lactic acid bacteria and physico-chemical properties of yoghurts. *Int. Dairy J.* **89**: 77-85. *https://doi.org/10.1016/j.idairyj.2018.09.003*.

Delgado-Fernández, P., Moreno, F. J., Corzo, N. and Nöbel, S. (2020). Physical properties of synbiotic yogurts as affected by the acidification rate. *Int. Dairy J.* **105**: 1-13. *https://doi.org/10.1016/j.idairyj.2020.104665*.

Dong, Y., Han, M., Fei, T., Liu, H. and Gai, Z. (2024). Utilization of diverse oligosaccharides for growth by *Bifdobacterium* and *Lactobacillus* species and their *in vitro* co-cultivation characteristics. *Int. Micro.* **27**: 941-952. *https://doi.org/10.1007/s10123-023-00446-x.*

Edwards, P. T., Kashyap, P. C. and Preidis, G. A. (2020). Microbiota on biotics: Probiotics, prebiotics and synbiotics to optimize growth and metabolism. *Am. J. Physiol. Gastrointest. Liver Physiol.* **3**: G281-G419. *https://doi.org/10.1152/ajpgi.00028.2020*.

Fara, A., Sabater, C., Palacios, J., Requena, T., Montilla, A. and Zarate, G. (2020). Prebiotic galacto-oligosaccharides production from lactose and lactulose by *Lactobacillus delbrueckii* subsp. *bulgaricus* CRL450. *Food Func.* **11**: 5875-5886. *Doi: 10.1039/d0fo00942c.*

Hutkins, R., Walter, J., Gibson, G. R., Bedu-Ferrari, C., Scott, K., Tancredi, D. J., Wijeyesekera, A. and Sanders, M. E. (2025). Classifying compounds as prebiotics-scientific perspectives and recommendations. *Nat. Rev. Gastro. Hep.* **22**: 54-70.

Iqbal, M. W., Riaz, T., Mahmood, S., Liaqat, H., Mushtaq, A., Khan, S., Amin, S. and Qi, X. (2023). Recent advances in the production, analysis, and application of galacto-oligosaccharides. *F. Rev. Int.* **38**: 5814-5843. *https://doi.org/10.1080/87559129.2022.2097255*.

Jr, R. J. A., White, A. K. and Jr, H. A. T. (2021). Identification of *Lactobacillus* strains capable of fermenting fructo-oligosaccharides and inulin. *Microorganisms* **9**: 1-11. *https://doi.org/10.3390/ microorganisms9102020.*

Kaewarsar, E., Chaiyasut, C., Lailerd, N., Makhamrueang, N., Peerajan, S. and Sirilun, S. (2023). Optimization of mixed inulin, fructooligosaccharides and galactooligosaccharides as prebiotics for

stimulation of probiotics growth and function. *Foods* **12**: 1-19. *https://doi.org/10.3390/ foods12081591.*

Kuerman, M., Bao, Y., Guo, Y. and Guo, M. (2020). Effects of prebiotic carbohydrates on the growth promotion and cholesterol-lowering abilities of compound probiotics *in vitro*. *LWT.* **118**: 1-28. *https://doi.org/10.1016/j.lwt.2019.108703*.

Maraz, A., Kovacs, Z., Benjamins, E. and Pazmandi, M. (2022). Recent developments in microbial production of high purity galacto oligosaccharides. *World. J. Micro. Biotech.* **95**: 1-10. *https://doi.org/10.1007/s11274-022-03279-4.*

Markowiak, P. and Œli¿ewska, K. (2017). Effects of probiotics, prebiotics and synbiotics on human health. *Nutrients* **9**: 1-30. *https://doi.org/10.1007/s11274-020-02961-9*.

Nunpan, S., Suwannachart, C. and Wayakanon, K. (2019). Effect of prebiotics-enhanced probiotics on the growth of *Streptococcus mutans*. *Int. J. Micro.* **7***:* 1-7. *https://doi.org/10.1155/2019/4623807*.

Peng, M., Tabashsum, Z., Anderson, M., Truong, A., Houser, A. K., Padilla, J. and Biswas, D. (2020). Effectiveness of probiotics, prebiotics and prebiotic like components in common functional foods. *Comp. Rev. Food Sci. Food Saf.* **4**: 1908-1933. *https://doi.org/10.1111/1541-4337.12565*.

Rehman, A., Arif, M., Sajjad, N., Al-Ghadi, M. Q., Alagawany, M., El-Hack, M. E. A., Alhimaidi, A. R., Elnesr, S. S., Almutairi, B. O., Amran, R. A., Hussein, E. O. S. and Swelum, A. A. (2020). Dietary effect of probiotics and prebiotics on broiler performance, carcass and immunity. *Poultry Sci.* **12**: 6946-6953. *https://doi.org/10.1016/j.psj.2020.09.043*.

Ruan, S., Xiang, S., Wu, W., Cao, S., Du, Q., Wang, Z., Chen, T., Shen, Q., Liu, Li., Chen, H., Weng, L., Zhu, H. and Liu, Q. (2020). Potential role of mTORC1 and the PI3K-Akt pathway in anti-acne properties of licorice flavonoids. *J. Functional Foods* **70**: 103968. *https://doi.org/10.1016/j.jff.2020.103968*.

Searns, J. B., Robinson, C. C., Qi, Wei., Yuan, Ji., Hamilton, S., Pretty, K., Donaldson, N., Parker, S. K. and Douinguez, S. R. (2019). Validation of a novel molecular diagnostic panel for pediatric musculoskeletal infections: Integration of the Cepheid Xpert MRSA/SA SSTI and laboratory-developed real-time PCR assays for clindamycin resistance genes and *Kingellakingae* detection. *J. Microbiol. Methods* **156**: 60-67. *https://doi.org/10.1016/j.mimet.2018.12.004*.

Sirbu, E., Dima, M. F., Tenciu, M., Cretu, M., Coada, M. T., Totoiu, A., Cristea, V. and Patriche, N. (2022). Effects of dietary supplementation with probiotics and prebiotics on growth, physiological condition and resistance to pathogens challenge in Nile Tilapia (*Oreochromis niloticus*). *Fishes* **5**: 1-21. *https://doi.org/10.3390/fishes7050273*.