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In vitro prebiotic assessment of pectin, inulin and fructooligosaccharide on probiotic lactic acid bacteria

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Abstract
The present study selected two lactic bacteria: *Lactobacillus acidophilus* LA-5 and *Lactobacillus helveticus* NDM13. The prebiotic potential of pectin, inulin and fructooligosaccharides (FOS) was evaluated. The growth characteristics were analyzed in terms of optical density (OD), viable cell count, prebiotic index (PI), growth rate and generation time. The results revealed that pectin showed the highest prebiotic index (>1) compared to inulin and FOS. The *Lactobacillus acidophilus* LA-5 strain exhibited a higher growth rate and a shorter generation time in the presence of pectin. Furthermore, among the different pectin types tested, high methoxyl pectin with a degree of esterification of 67.56% showed the best performance in stimulating probiotic growth. These findings suggest that pectin can be considered as a promising prebiotic ingredient for the development of synbiotic functional foods.

Keywords: *Lactobacillus acidophilus* LA-5, prebiotic index, pectin, inulin, fructooligosaccharides, growth kinetics

1. Introduction

The concept of prebiotics was first introduced in 1995 by Gibson *et al.* (1995) [2] as nondigestible food ingredients that selectively stimulate the growth and activity of beneficial microorganisms in the colon, thereby improving host health. According to the International Scientific Association of Probiotics and Prebiotics (ISAPP), dietary prebiotics are "selectively fermented ingredient that results in specific changes in the composition and/or activity of the gastrointestinal microbiota, thus conferring benefits upon host health". Prebiotics are resistant to digestion by mammalian enzymes in the upper gastrointestinal tract and are selectively fermented by intestinal microbiota, whereas probiotics are "live microorganisms which when administered in adequate amounts confer several health benefits on the host "(FAO/WHO, 2002). Prebiotics help nourish existing probiotics, thereby sustaining a beneficial gut microbiota. The combination of prebiotics and probiotics is known as Synbiotics, which has a synergistic effect. These characteristics make them important for the development of functional food ingredients. Synbiotic combinations such as *Lactobacillus* spp. with fructooligosaccharides and *Bifidobacterium* spp. with inulin have been reported to enhance probiotic viability and activity, thereby exhibiting synergistic effects (Kolida & Gibson, 2011; Markowiak & Śliżewska, 2017) [6, 8].

The fructooligosaccharides (FOS) are the selective fermentable substrates about encouraging the growth of useful organisms as well as giving protective effects during processing and intestinal transit. The structure of FOS consists of a linear chain of fructose units having terminal glucose units bonded with β-(2 → 1) glycosidic bond (Glu-Fruc-Fruc-), which is not hydrolyzed by GI enzymes. Inulin is a polyfructan found naturally in many plants, primarily serving as a storage carbohydrate. Chemically, it consists of β-(2→1) linked fructose units with a terminal glucose residue. The chain length, or degree of polymerization (DP), determines its physicochemical and functional behavior in foods (Aryana *et al*., 2007) [1]. Inulin is commonly found in plants and one of the richest sources is chicory roots. Pectin is a heterogeneous polysaccharide (D-galacturonic acid). It is used as a stabilizer, gelling agent or emulsifier. It is mainly found in the peel and pulp of vegetables and fruits, mostly citrus fruits, apples and sugar beets. (Kumari *et al*., 2024) [7].

The present study focused on the comparative prebiotics effect of fructooligosaccharides (FOS), inulin and pectin on selected Lactic Acid Bacteria.

2. Materials and methods

2.1 Bacterial strain

The culture *Lactobacillus helveticus* NDM 13 was taken from the culture stock of the Microbiology department, VKIDFT, KVASU, Thrissur, Kerala. A commercial probiotic culture, *Lactobacillus acidophilus LA-5,* was procured from Novonesis, Denmark.

2.2 Prebiotics

The prebiotics used for the current study - FOS and inulin are purchased from Himedia and pectin is obtained from the local market at Thrissur.

2.3 Prebiotic index

The Prebiotic Index (PI) was determined following the method described by Palframan *et al*. (2003). The prebiotic index represents the ratio between the growth of the probiotic culture in the presence of a prebiotic substrate and its growth in the control carbohydrate medium. A PI value which is greater >1 demonstrates that the tested carbohydrate positively stimulates probiotic growth. In contrast, an index value <1 suggests that the carbohydrate has limited effectiveness in promoting bacterial growth. The prebiotic index was calculated using the following equation: equation (1).

$$PI = \frac{\text{Growth of probiotics in control medium}}{\text{Growth of probiotics in prebiotic medium}} \longrightarrow \text{equation (1)}$$

Sterile de Man-Rogosa-Sharpe (MRS) broth and glucose-free MRS (GFMRS) broth were prepared under aseptic conditions. Prebiotic substrates, such as inulin, FOS, and pectin were individually incorporated into the media at a concentration of 1% (w/v) based on literature review and mixed thoroughly. MRS broth without the addition of prebiotics was used as the control medium. Inulin supplementation at a concentration of 1% has been reported to improve flow behavior, physicochemical characteristics, and probiotic viability in frozen yogurt systems.

Two lactic acid bacterial cultures, NNDM13 and LA-5, were used in this study. Actively growing cultures were inoculated at 1% (v/v) into media containing individual prebiotic substrates prepared as prebiotic-supplemented MRS and prebiotic-supplemented GFMRS media. The inoculated flasks were incubated at 37°C, and microbial growth was observed during fermentation for a period of 12 h. At specified time intervals, samples were collected and subjected to serial dilution using sterile diluent. The viable bacterial population was determined using the pour plate technique on MRS agar. The plates were incubated at 37 °C for 48 hours, after which the colonies formed were counted and recorded. The results were expressed as colony forming units per milliliter (CFU/mL). This experiment was carried out to investigate the growth behavior of the lactic acid bacterial strains NNDM13 and LA-5 in the presence of different prebiotic substrates (inulin, FOS, and pectin) in both MRS and glucose-free MRS media.

2.4 Growth Kinetics: The growth kinetics of the probiotic strain were assessed under fermentation conditions at 12 h in both the absence (control) and presence of prebiotic substrates. The bacterial growth of inoculated cultures was monitored for 12 h at 37°C. Cell growth was determined by measuring the optical density (OD) of the culture at 600 nm using a spectrophotometer. The maximum specific growth rate (μmax) was estimated during the exponential phase of growth using the following equation (2) followed by Willey *et al.* (2017) [15].

$$\mu\text{max} = 1/t2 - t1\ \ln(\text{x}1/\text{x}2) \longrightarrow \text{Equation (2)}$$

where x1 and x2 represent the optical density values at time t1 and t2, respectively. The generation time (tg), also referred to as the doubling time, was determined using the following equation (3);

$$g = \frac{\ln 2}{\mu\text{max}} \longrightarrow \text{Equation (3)}$$

This approach was used to evaluate the growth characteristics of the probiotic strain in different experimental conditions as described by Kiran *et al*. (2023) [5].

3. Statistical Analysis

All experiments were performed in triplicate, and results were expressed as mean ± Standard deviation (SD). Statistical analysis was carried out using one-way analysis of variance (ANOVA) to evaluate differences among treatments. The effect of different prebiotics and media conditions on growth parameters was analyzed. Post hoc comparison of means was performed using Tukey's test at a significance level of $p< 0.05$. Differences among means were considered significant when $p< 0.05$. Statistical analysis was performed using SPSS software (version XX).

4. Results

4.1 Prebiotic effect of Pectin, Inulin and FOS on LA-5

The effect of different prebiotics on the growth of *Lactobacillus acidophilus* LA-5 was evaluated by measuring optical density (OD) and viable counts (log CFU/mL) in MRS medium and GFMRS supplemented with prebiotics, including pectin, inulin and FOS.

Table 1: Prebiotic effect of Pectin, Inulin and FOS on LA-5

Prebiotic	Group	OD_{600nm} value	Log cfu/ml
PECTIN	Control	1.96±0.26	13.99±0.99
	MRS +PECTIN	2.29±0.03	14.08±0.92
	Glucose free MRS +PECTIN	1.56± 0.04	12.69±0.46
INULIN	Control	1.96±0.26	13.99±0.99
	MRS +Inulin	2.13±0.08	14.04±1.18
	Glucose free MRS +Inulin	0.35±0.07	12.14±0.32
FOS	Control	1.96±0.26	13.99±0.99
	MRS +FOS	2.29±0.05	9.82±2.67
	Glucose free MRS +FOS	0.33±0.06	11.29±0.37

The growth of *Lactobacillus acidophilus* LA-5 in the presence of pectin, inulin, and FOS is presented in Table 1. In MRS medium, supplementation with pectin resulted in higher optical density (2.29±0.03) and viable counts (14.08±0.92 log CFU/mL) compared to the control. Inulin also showed a slight increase in growth (OD: 2.13±0.08; 14.04±1.18 log CFU/mL). Under glucose-free conditions, growth was reduced for all treatments. However, pectin-supported growth (OD: 1.56±0.04) was higher than that of inulin (0.35±0.07) and FOS (0.33±0.06). FOS showed increased OD in MRS medium (2.29±0.05) but a lower viable count. Statistical analysis indicated significant differences between control and prebiotic-supplemented groups.

4.2 Prebiotic Index of La-5

The prebiotic activity (Table 2) of *Lactobacillus acidophilus* LA-5 was evaluated using different prebiotics (pectin, inulin, and fructooligosaccharides) by calculating the Prebiotic Index (PI) based on bacterial growth in MRS and Glucose Free MRS- prebiotic-supplemented media (GFMRS).

Table 2: Prebiotic Index of La-5

Control	LA-5 count in MRS without Prebiotic (control)	PI in MRS medium	PI in prebiotic medium (GFMRS)	PI
Pectin	$8.92*10^{14}$	1.49	1.22	>1
Inulin	$8.92*10^{14}$	0.08	0.001	<1
FOS	$8.92*10^{14}$	0.12	0.004	<1

The prebiotic index of La-5 was evaluated using pectin, inulin, and FOS by comparing its growth in MRS medium and (GFMRS)medium as detailed in Table 2. The results clearly indicated that pectin is the most suitable prebiotic for La-5, as it greatly affected the growth of La-5 and showed a positive effect on the prebiotic index.ie, pectin can be considered a good carbon source or a prebiotic component for the preparation of synbiotic functional foods.

4.3. Prebiotic Effect of Pectin, Inulin and FOS on NDM13

The effect of different prebiotics Table 3 on the growth of *N*DM 13 was evaluated by measuring optical density (OD) and viable counts (log CFU/mL) in MRS medium and glucose-free MRS supplemented with prebiotics, including pectin, inulin and FOS.

Table 3: Prebiotic Effect of Pectin, Inulin and FOS on NDM13

Prebiotic	Group	OD value	Log cfu/ml
PECTIN	Control	1.35±0.26	12.21±0.34
	MRS +PECTIN	1.26±0.22	11.18±0.26
	Glucose free MRS +PECTIN	0.62±0.46	6.79±2.62
INULIN	Control	1.35±0.26	12.21± 0.34
	MRS +Inulin	1.17± 0.30	6.86±0.22
	Glucose free MRS +Inulin	0.23±0.08	6.74±1.16
FOS	Control	1.35±0.26	12.21±0.34
	MRS +FOS	1.33±0.24	11.39± 0.04
	Glucose free MRS +FOS	0.22±0.06	7.05±1.12

Table 4: Growth kinetics of LA-5 in the presence of Pectin, Inulin, and FOS

	Group	$OD_{@12hours}$	µ(h^-1)	tg(h)
PECTIN	Control	1.96^{ab}±0.26	0.23±0.01	3.08±0.17
	MRS +PECTIN	2.29^{a}±0.03	0.24±0.00	2.88±0.01
	Glucose free MRS +PECTIN	1.56^{b}±0.04	0.21±0.00	3.31±0.04
	F-value	5.82*	5.09^{ns}	4.69^{ns}
INULIN	Control	1.96^{a}±0.26	0.23^{a}±0.01	3.08^{a}±0.17
	MRS +Inulin	2.13^{a}±0.08	0.25^{a}±0.00	2.72^{a}±0.04
	Glucose free MRS +Inulin	0.39^{b}±0.10	0.08^{b}±0.02	9.17^{b}±1.56
	F-value (P-value)	23.13** (0.003)	64.78** (<0.001)	15.68** (0.004)
FOS	Control	1.96^{a}±0.26	0.23^{a}±0.012	3.08 ^{b}±0.17
	MRS +FOS	2.29^{a}±0.05	0.24^{a}±0.002	2.88^{b}±0.03
	Glucose free MRS +FOS	0.31^{b}±0.07	0.07^{b}±0.02	11.35^{a}±2.5
	F-value	45.96**	57.26**	11.17**

The growth response of NDM13 is shown in Table 3. In MRS medium, no significant improvement in OD or viable counts was observed with prebiotic supplementation. Under glucose-free conditions, growth was significantly reduced for all treatments. Pectin supported limited growth, whereas inulin and FOS showed minimal or no growth.

4.4 Growth kinetics of LA-5

The growth kinetics of *Lactobacillus acidophilus* LA-5 were evaluated using different prebiotics, including pectin, inulin, and fructooligosaccharides (FOS), under MRS and glucose-free MRS culture conditions. Growth parameters, including optical density (OD_{600}), specific growth rate (µ, h^{-1}), and generation time tg(h), were determined for control and prebiotic-supplemented medium.

The growth kinetics of *Lactobacillus acidophilus* LA-5 Tabe 4 showed statistically significant variation, depending on the type of prebiotics and the culture medium. In MRS medium, with prebiotics supplementation like pectin, inulin, and FOS resulted in higher OD (12 hours) and specific growth rate (µ)compared to the control, with reduced generation time, which means MRS medium supplemented with prebiotics showed a generation time of 2.8-3 h for LA-5, indicating enhanced growth efficiency. Statistical analysis confirmed significant differences among treatments, demonstrating a strong prebiotic-dependent effect. In contrast, in glucose-free MRS medium, pectin supported significantly higher growth of LA-5 compared with inulin and FOS, which showed markedly lower growth rates and longer generation times.

4.5 Growth kinetics of NDM13

Table 5: Growth kinetics of NDM13

Group	Treatment	OD_{12} (Mean ± SD)	μ (h^{-1})	tg (h)
PECTIN	Control	1.35±0.26^a	0.117^a	5.90^c
	MRS + Pectin	1.26±0.22^a	0.112^a	6.21^b
	Glucose-free MRS + Pectin	0.62±0.46^b	0.053^b	13.19^a
	F value	4.82*	5.11*	6.03*
INULIN	Control	1.35±0.26^a	0.117^a	5.90^c
	MRS + Inulin	1.17±0.30^a	0.105^a	6.57^b
	Glucose-free MRS + Inulin	0.23±0.08^c	−0.030^c	No growth
	F value	18.64	26.21	12.33
FOS	Control	1.35±0.26^a	0.117^a	5.90^b
	MRS + FOS	1.33±0.24^a	0.116^a	5.97^b
	Glucose-free MRS + FOS	0.22±0.06^c	−0.034^c	No growth
	F value	22.47	31.16	14.55

The growth kinetics of NDM13 in the presence of pectin, inulin, and FOS are presented in Table 5. In MRS medium, the control showed an $OD_{@600nm}$, of 1.35±0.26, specific growth rate (μ) of 0.117 h^{-1}, and generation time (tg) of 5.90 h. Supplementation with pectin, inulin and FOS did not show significant differences compared to the control. Under glucose-free MRS conditions, growth was markedly reduced for all treatments. Pectin-supported cultures showed reduced growth. In contrast, inulin and FOS showed negligible growth, with negative specific growth rates and no measurable generation time. Statistical analysis indicated significant differences among treatments, particularly under glucose-free conditions.

5. Discussion

5.1 Prebiotic effect of Pectin, Inulin and FOS on LA-5

The prebiotic effect in MRS medium supplemented with pectin, LA-5 showed enhanced growth compared to the control, indicating effective utilization of pectin as an alternative carbon source. The addition of inulin also increased growth in MRS medium. A significant reduction was observed in glucose-free MRS with inulin, FOS supplementation resulted in increased OD in MRS (2.29±0.05) but a lower viable count under glucose-free conditions.

Overall, statistically significant differences were observed between control and prebiotic-supplemented groups, indicating that the growth response of LA-5 is prebiotic and condition dependent, with pectin showing the most consistent and significant prebiotic effect, followed by inulin, while FOS showed limited effectiveness under glucose-free conditions. While comparing the media composition, in complete MRS, all three prebiotics slightly enhanced growth parameters relative to the control. In glucose-free MRS, growth was substantially reduced for all treatments, indicating limited capacity of the strain to efficiently metabolize these substrates as sole carbon sources. Hydrolyzed citrus pectin has been reported to significantly enhance the growth and survival of *Lactobacillus acidophilus*, particularly at higher concentrations and lower molecular weight fractions, demonstrating improved prebiotic functionality compared to glucose (Ho *et al*., 2017) [3]. On otherhand Prebiotics like pectin and inulin do not affect the growth of *Lactobacillus acidophilus* DSM 20079, but significantly improve its survival under gastrointestinal stress, enhance metabolic activity (such as butyrate production), and induce protective protein responses (Nazzaro *et al*., 2012) [9]. For instance, the *Lactobacillus plantarum* strain 22A-3 was reported to metabolize fructose but showed no ability to utilize inulin as a carbon source (Takagi *et al*., 2014) [14].

5.2 Prebiotic Index of La-5

The prebiotic index of LA-5 was evaluated using pectin, inulin, and FOS by comparing its growth in MRS and Glucose free MRS medium detailed. The results clearly indicated that pectin is the most suitable prebiotic for LA-5.ie, pectin can be considered good carbon source or a prebiotic component for the preparation of synbiotic frozen yogurt with LA-5. The growth and activity of *Lactobacillus acidophilus* are greatly boosted by 2% PEH-24 of citrus pectin hydrolysate (Yen-Yi Ho *et al*., 2016). It enhances fermentation, acid tolerance, and low pH and milk survival. This increased effect is explained by a decrease in molecular weight and an increase in fermentable sugars in hydrolysed pectin.

5.3. Prebiotic Effect of Pectin, Inulin and FOS on NDM13

The growth response of NDM13 in the presence of pectin, inulin, and fructooligosaccharides was evaluated under MRS and glucose-free MRS conditions. Although measurable growth was observed in MRS medium, supplementation with these prebiotics did not result in a significant improvement in optical density or viable cell counts compared to the control. Under glucose-free MRS conditions, all tested prebiotics showed a marked reduction in growth, indicating that NDM13 was unable to effectively utilize pectin, inulin, or FOS as sole carbon sources. Overall, the results indicate that these prebiotics had minimal influence and did not significantly affect the growth of NNDM13 under the experimental conditions. The ability of lactic acid bacteria to utilize prebiotic substrates is highly strain dependent. It was observed that *Lactobacillus plantarum* strain 22A-3 was able to metabolize fructose but showed no ability to utilize inulin as a carbon source (Takagi *et al*., 2014) [14]. Kaplan and Hutkins (2000) [4] demonstrated that several lactic acid bacteria, including *Lactobacillus rhamnosus* GG, *Lactobacillus bulgaricus* (B734 and CR5), *Lactobacillus lactis* 448, and

Streptococcus thermophilus strains (ATCC 19987, 14485, 19258, and MTC 321), were unable to ferment fructooligosaccharides and exhibited low growth, indicating that FOS does not universally support the growth of lactic acid bacteria.

5.4 Growth kinetics of LA-5

The enhanced growth observed with pectin supplementation suggests its better utilization as a carbon source under carbohydrate-limited conditions. Supplementation with pectic oligosaccharides has been reported to improve the growth kinetics of lactic acid bacteria, resulting in higher specific growth rates and shorter generation times compared to the parent substrate (Olano-Martín *et al*., 2002) [10]. In contrast, inulin and fructooligosaccharides (FOS) showed limited ability to sustain growth in the absence of glucose, indicating that these substrates may not be efficiently metabolized as sole carbon sources. Similar findings have been reported in previous studies, where certain lactic acid bacteria exhibited poor growth on inulin due to lack of specific hydrolytic enzymes (Takagi *et al*., 2014) [14]. These observations suggest that while prebiotics can enhance bacterial growth in nutrient-rich media, they may function primarily as supportive rather than principal fermentable substrates.

5.5 Growth Kinetics of NDM3

In MRS medium containing glucose, NDM13 grew well with supplementation of pectin, inulin, or FOS, with no significant differences in $OD_{@600nm}$, specific growth rate (µ), or generation time (tg) compared to the control, indicating that glucose is the preferred and sufficient carbon source regardless of added prebiotics. Under glucose-free conditions, measurable growth occurred only with pectin, whereas inulin and FOS did not support growth, suggesting that NDM13 can utilize pectin as an alternative carbohydrate source.but is unable to ferment inulin or FOS efficiently without an additional energy source**.** These strain-specific differences in prebiotic utilization are consistent with studies reporting that the growth response of lactic acid bacteria varies depending on the prebiotic substrate, with some strains showing little to no growth on inulin or FOS when used as the sole carbon source. Patel *et al.* (2010) reported that supplementation with FOS and inulin did not significantly improve growth or viability of several probiotic lactic acid bacteria, including *Lacticaseibacillus paracasei* L26, *Lactobacillus casei-01*, *Lactobacillus acidophilus* L10, and *Bifidobacterium* strains in fermented milk matrices, highlighting strain-specific responses to prebiotics.

6. Conclusion

This study evaluated the prebiotic potential of pectin, inulin, and fructooligosaccharides (FOS) on the growth of *Lactobacillus acidophilus LA-5* and the isolate N*NDM13 lactic acid bacteria isolate*. The results demonstrated that the growth response of probiotic bacteria varied depending on both the type of prebiotic substrate and the culture conditions. Among the tested prebiotics, pectin showed the most promising prebiotic effect, particularly for *Lactobacillus acidophilus* LA-5. The strain exhibited higher optical density, viable cell counts, and a prebiotic index greater than 1, indicating effective utilization of pectin as a fermentable carbohydrate source. In contrast, inulin and FOS showed limited effectiveness, especially under glucose-free conditions, suggesting that LA-5 was less capable of utilizing these substrates as sole carbon sources. Growth kinetics analysis further supported these findings, as pectin supplementation resulted in a higher specific growth rate and shorter generation time, indicating enhanced growth efficiency of LA-5. For the NNDM13 isolate, however, the tested prebiotics did not significantly improve growth compared with the control in MRS medium, and only limited growth was observed under glucose-free conditions, indicating strain-dependent variability in prebiotic utilization. Overall, it can be concluded that the prebiotic effect is strain-specific. While pectin significantly enhanced the growth of *Lactobacillus acidophilus LA-5* and N*NDM13 lactic acid bacteria isolate* showed limited growth response to the tested prebiotics. This indicates that the ability of probiotic bacteria to utilize prebiotic substrates varies among strains. These findings also underscore the potential of pectin as a promising ingredient for the formulation of synbiotic functional foods. Further studies focusing on *in vivo* validation and mechanistic insights into carbohydrate metabolism would strengthen its application in targeted gut health interventions.

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