Characterizing Xylooligosaccharide (XOS) for its prebiotic properties- an in vitro study

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Abstract: This study was undertaken to explore the prebiotic potential of XOS. Acid tolerance, bile resistance and fermentability of XOS to produce short chain fatty acids (SCFA) were determined using HPLC analysis, growth of the selected bacterial strains was determined using spectrophotometer. No degradation of XOS was observed on its exposure to bile at 0h, 1.5h and 3h with bile concentration 0.5%, 1% and 1.5%. XOS recovery was observed to be 100% on its exposure to pH 1.5, 2 and 3 at 0h. At 1.5h it was found to be 98.4%, 98.9% and 97.9% at 1.5pH, 2pH and 3pH respectively and 96.2%, 97.3% and 96.3% on its exposure to 1.5pH, 2pH and 3pH respectively at 3h. Growth of *Lactobacillus plantarum* and *Bifidobacterium adolescentis* was higher (OD 0.71) and (OD 0.75) up to 2% when compared to glucose with ($p \leq 0.01$), whereas, growth of *Escherichia coli*(OD 0.4) was restricted upon addition of XOS when compared to glucose ($p \leq 0.01$). Acetate was produced the most, followed by Propionate and Butyrate.

Keywords: XOS, SCFA, acid tolerance, bile resistance.

1. INTRODUCTION

The concept of prebiotic was introduced around 20 years ago, despite several revisions to the original definition which was introduced in 1995 by Glenn Gibson and Marcel Roberfroid, the scientific community continued to debate what it means to be a prebiotic. (R.W. Hutkins et al, 2016). Prebiotics are defined as a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microflora that confers benefits upon host well being and health (GR Gibson et al, 2004). It is also defined as a non digestible compound that, through its metabolization by microorganisms in the gut, modulates the composition and/or activity of the gut microbiota, thus conferring a beneficial physiologic effect on the host (LB Bindels et al, 2015).

Several studies have confirmed that prebiotics are a valid approach to the dietary manipulation of the colonic micro flora and thereby improved glycemic, lipemic, inflammatory biomarkers and increased the production of short chain fatty acids (Roycroft C.E. et al, 2001, Sheth M et al, 2016, Lin S.H. et al, 2016).

The prebiotic potential of XOS have been explored the least whereas, inulin, FOS, resistance starch etc. have been explored to a great extent. Therefore, this in vitro study was intended to explore the prebiotic potential of XOS with regards to acid tolerance, bile resistance, growth of probiotic bacteria such as *Lactobacillus Plantarum* and *Bifidobacterium Adolescentis* and production of SCFA namely, acetate, butyrate and propionate.

2. METHODS AND MATERIALS

2.1. MATERIALS FOR BILE RESISTANCE AND ACID TOLERANCE TEST OF XOS

Commercial XOS derived from corn cobs were purchased from Hangzhou Focus Corporation. (Hangzhou, China) and was 95% pure, Ox bile and hydrochloric acid procured from Sigma.

2.2. BILE RESISTANCE TEST OF XOS

Ox bile (1g) was dissolved in 100ml DI water and stirred well till it dissolved. Bile solution was made up to bile level 0.5%, 1% and 1.5% using Ox bile (Sigma) and 5g XOS was added to the bile solutions at room temperature. These solutions were used to study bile resistance of XOS at 0h, 1.5h and 3h. The samples were filtered and 20 micro litre each were used for the HPLC analysis. This method was modified in house with reference to (R. M. Duar, 2011).

2.3. ACID TOLERANCE TEST OF XOS

XOS (5g) were dissolved in 100ml DI water and stirred well till it dissolved. X ml (QS) Hydrochloric acid solution was added to the solution to adjust pH = 1.5, 2.0 and 3.0. These solutions were used to study acid tolerance of XOS at 0h, 1.5h and 3h. The samples were filtered and 20 micro litre each were used for the HPLC analysis. This method was modified in house with reference to (R. M. Duar, 2011).

2.4. MATERIALS FOR DETERMINATION OF PREBIOTIC EFFECT OF XOS ON *L. Plantarum, B. Adolescentis, E. Coli* AND SCFA ANALYSES USING HPLC

Commercial XOS derived from corn cobs were purchased from Hangzhou Focus Corporation. (Hangzhou, China) and was 95% pure. The degrees of polymerization of the XOS mixture ranged from xylobiose to xylohexaose. All chemicals were purchased from Sigma-Aldrich, India. Bacterial culture for *Lactobacillus Plantarum* strain was purchased from MTCC repository, *Bifidobacterium Adolescentis* strain from National collection of Dairy culture, National Institute of Dairy Research, Karnal. *Escherichia coli* were isolated from sewage at Institute of Science, Nirma University.

2.5. BACTERIAL STRAINS

Lactobacillus plantarum strain (MTT2621), *Bifidobacterium adolescentis* strain (NCDC236) were used in the present study. The bacterial pathogen used was *Escherichia coli*.

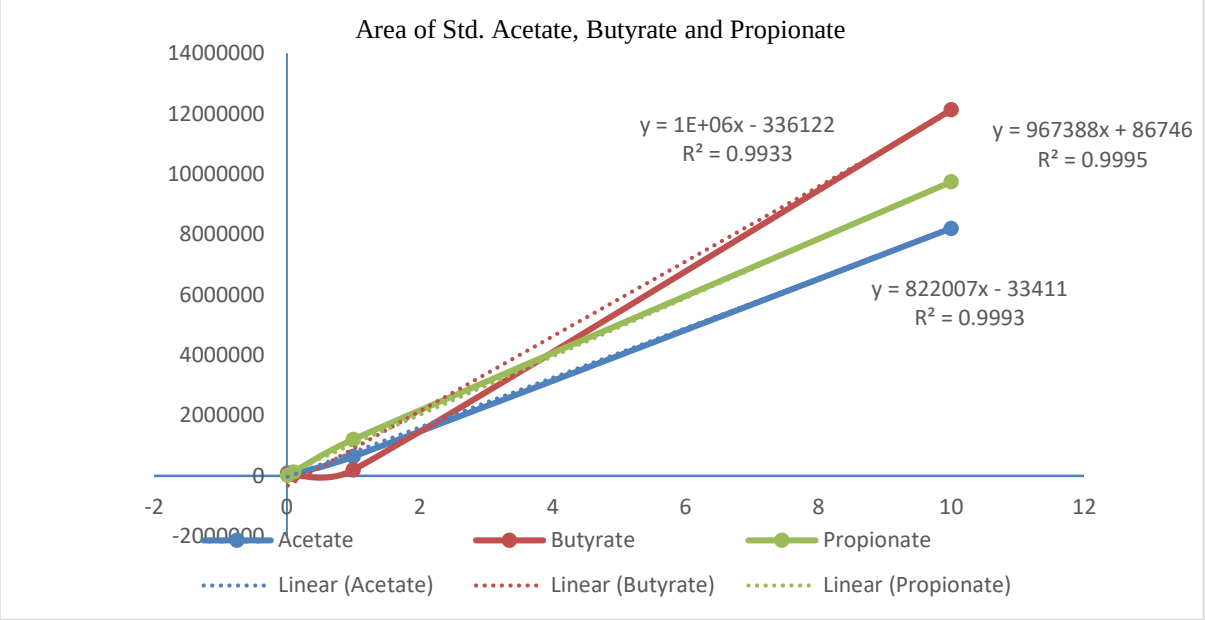
2.6. PREBIOTIC EFFECT OF XOS ON *L. Plantarum, B. Adolescentis* AND *E. Coli*

Lactobacillus plantarum was grown in MRS broth in anaerobic jar, *Bifidobacterium adolescentis* in MRS broth along with 0.05% cysteine in anaerobic jar and *Escherichia coli* in Luria–Bertani (LB) broth at 37°C for 24 h. After 24 h, each bacterium was allowed to grow with XOS concentration 0.5%, 1%, 2%, 3% and 4%. The bacteria were grown on their respective media such as MRS agar and Luria broth without XOS as negative control. 10%v/v inoculation was added from the active culture having OD between 0.08 and 0.1 at 620nm. These were then incubated at 37°C for 24 h. After 24 h, readings were taken in Spectrophotometer (Agilent, model no: carry 60) at 620nm.Prior to each OD measurement the flasks were carefully shaken. The concentration of XOS which gave maximum OD for each bacterium was further chosen for SCFA analysis using HPLC. All measurements were performed in duplicates.

2.7. SCFA ANALYSES USING HPLC

To evaluate the efficiency of the fermentation of XOS by *Lactobacillus plantarum* strain (MTT2621), *Bifidobacterium adolescentis* strain (NCDC236) and E. Coli, HPLC was performed. Acetic, Butyric and propionic acids, products of the XOS fermentation, can be detected in the growth medium and quantified by HPLC. The first step of the experimental set-up was choosing the appropriate column for efficient separation of the analytes. The column Phenyl hexyl, 100 × 4.6 mm (Agilent technologies, USA) was chosen as it was prepared for separation of small polar compounds such as short-chain fatty acids. This HPLC consists of UV210 detector (Shimadzu, Kyoto, Japan) connected to a recorder. Separation of the analytes took place in the aforementioned Phenyl hexylcolumn. The peaks for analysis were obtained in the computer software connected to it.

24 hour old culture was centrifuged at 7,000 rpm for 5 mins. Supernatant was diluted (1:1) in buffer containing 2.5pH water using H_3PO_4. 10mg/ml of standards of acetate, butyrate and propionate (HPLC grade, Sigma) were run at 1mg/ml flow rate in the HPLC till 15 minutes and 20µl of samples/standard were injected in phenyl hexyl column (Agilent). Detection wavelength was 210 nm and recording range was set to 0.2 absorbance unit's full scale. Prior to use, the mobile phase and samples were filtered through 0.2un filter prior to injecting the samples in the column. Fig shows the area of standard Acetate, Butyrate and Propionate. Area was calculated for the standards and SCFA samples were calculated based on the area of standards.



2.8. STATISTICAL ANALYSIS

The HPLC analysis of XOS recovery on bile resistance and acid tolerance were conducted in duplicates for each of the samples at 0.5%, 1% and 1.5% bile concentration and 1.5pH, 2pH and 3pH respectively. The effect of incubation period on recovery of XOS was conducted at 0h, 1.5h and 3h. Growth of bacteria was conducted in triplicates and OD was measured for each samples. Production of SCFA was also analysed using HPLC. Data were collected and analyzed by using one-way analysis of variance (ANOVA). The significant differences between tests were set at $p \leq 0.05$. All statistical analyses were performed using Microsoft office excel 2007.

3. RESULTS AND DISCUSSION

3.1. BILE RESISTANCE TEST OF XOS

No degradation of XOS was observed on exposure of XOS to bile at 0h, 1.5h and 3h with bile concentration 0.5%, 1% and 1.5%. The tests were carried out in duplicates. A study on pH stability of prebiotic non-digestible wheat bran-derived arabinoxylooligosaccharides (AXOS), xylooligosaccharides (XOS)-and chicory root inulin-derived fructooligosaccharides (FOS) were compared. Decomposition was revealed at alkaline pH (pH 11.0) for all three preparations tested. The short chain oligosaccharides, XOS and FOS were more sensitive to alkaline decomposition than were the longer chain AXOS, the latter being the result of the higher abundance of reducing ends in short chain oligosaccharide preparations (Courtin M.C et al, 2009).

3.2. ACID TOLERANCE TEST OF XOS

As shown in table 3.1, XOS recovery was observed to be 100% on its exposure to pH 1.5, 2 and 3 at 0h. At 1.5h recovery of XOS was found to be 98.4%, 98.9% and 97.9% at 1.5pH, 2pH and 3pH respectively. XOS recovery was 96.2%, 97.3% and 96.3% on its exposure to 1.5pH, 2pH and 3pH respectively at 3h. The tests were carried out in duplicates.

At pH 2.0 and 3.0, hydrolysis of oligosaccharide linkages took place, with FOS being the most acid-sensitive component (Courtin MC et al, 2009). Recoveries were 100%, 91% and 113% for the supplemented muffin, cookie and nutrition bar, respectively at 3.5 pH. For the breakfast cereal, only 47% of the supplemented FOS remained after extrusion at optimal conditions (170 rpm and 140 °C) (Duar RM, 2011). Whereas, recoveries of Inulin at pH 3.5 were 106%, 103% and 107% and 126% obtained from the supplemented extruded cereal, nutrition bar, sports drink and muffins, respectively (Duar RM, 2011).

Another study on evaluation of the prebiotic effects of citrus pectin hydrolysate (PEH), it was found that when pH was reduced to 3.2, populations of the tested probiotics did not decrease significantly ($p > 0.05$) for all treatments. The tested probiotics showed significantly higher acid tolerance and survival populations in the media supplemented with PEH than glucose. This indicated that PEH should contain some oligosaccharides which assisted the probiotics in acid tolerance and survival ability, while glucose did not (Yen YH et al, 2017).

Table 3.1: XOS recovery at different levels of pH

pH	0h	1.5h	3h
1.5pH	100%	98.41%	96.29%
2pH	100%	98.94%	97.32%
3pH	100%	97.93%	96.39%

Cummings JH et al, 2001 reviewed on the digestibility of Inulin and Oligofructose and found an average recovery of 88% in human upper intestine.

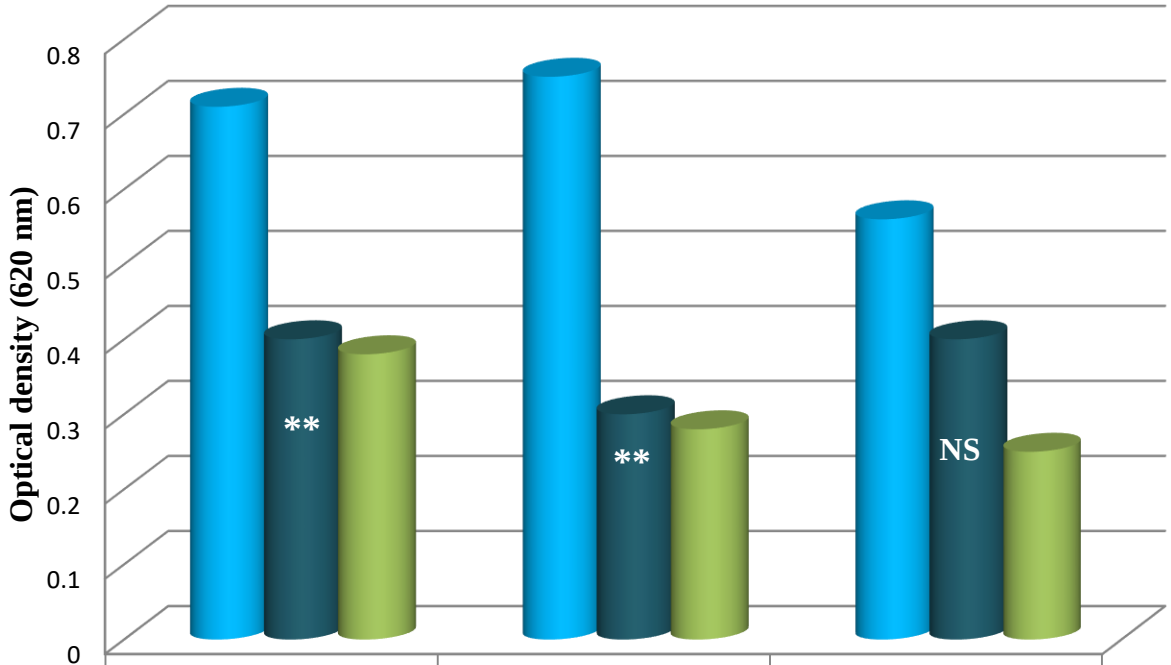
There is little available information in the literature on bile resistance, acid tolerance properties of XOS *in vitro*.

3.3. PREBIOTIC EFFECT OF XOS ON THE GROWTH OF *L. Plantarum, B. Adolescentis* AND *E. Coli*

As seen in figure 3.3.2, the growth of *Lactobacillus plantarum (LP)* and *Bifidobacterium adolescentis (BA)* were higher at 0.5%, 1% and 2% of XOS addition. For *Escherichia coli (E.coli)* the growth gradually decreased as the concentration of XOS increased from 0.5% to 2%. Since 0.5%, 1% and 2% levels of XOS concentration gave better or almost equivalent growth of *Lactobacillus plantarum (LP), Bifidobacterium adolescentis(BA)* and reduced the growth of *Escherichia coli (E. coli).* Therefore, 0.5%, 1% and 2% levels of XOS concentration samples were chosen for production of short chain fatty acids (SCFA) and its analysis.

Fig 3.3.2.shows mean growth of *Lactobacillus plantarum* was more with 0.5% and 1% XOS concentration at $p \leq 0.01$, growth of *Bifidobacterium adolescentis* was seen to be same with 0.5% and 2% XOS concentration at $p \leq 0.01$ and growth of *Escherichia coli* was the least with 1% XOS.

Fig: 3.3.2. Mean growth of *Lactobacillus plantarum, Bifidobacterium adolescentis* and *Escherichia coli* with 0.5%, 1% and 2% levels of XOS



	XOS 0.5%	XOS 1%	XOS 2%
O.D. (LP)	0.71	0.75	0.56
O.D.(BA)	0.4	0.3	0.4
O.D.(EC)	0.38	0.28	0.25

Significant at *p≤0.05, **p≤0.01, *** p≤0.001

A study on functional properties of commercial prebiotics showed the increase in cell density of *L. paracasei 1195* grown on Raftilose P95, Inulin-S, and Raftiline HP were significantly higher (p≤0.05) than for glucose. *B. bifidum NCI* had a significantly higher (p≤0.05) increase in cell density when grown on NutraFlora P-95 and Raftilose P95 than on glucose. Also, the increase in cell densities of *L. plantarum 4008* and *L. acidophilus 33200* were significantly larger (p≤0.05) for purified GOS than for glucose (Huebner J. Et al, 2007).

An in vitro study investigated the potential prebiotic effect of natural (NS) and blanched (BS) almond skins, the latter being a by-product of the almond-processing industry. Their study concluded that dietary fibre from almond skins altered the composition of gut bacteria and almond skins resulting from industrial blanching could be used as potential prebiotics (Mandalari G. et al, 2009).

A study on the prebiotic activity of XOS obtained from corncob and reagent grade xylan were tested in *L. brevis, L. plantarum, L. acidophilus, L. rhamnosus* cultures, and in a co-culture with *Escherichia coli* as a challenge microorganism to prove the bacteriostatic activity of lactobacilli strains. Xylooligosaccharides stimulated *L. brevis* and *L. plantarum* growth: these microorganisms grew faster than the other lactobacilli strains. *L. acidophilus* grew better in the presence of XOS and maintained the absorbance of the culture. In the co-culture in presence of both XOS the challenge microorganism did not grow; lactobacilli colonies appeared in MRS agar. No colonies of *E. coli* grew in EMB plaques (Pedraza L. et al, 2014).

3.4. SCFA PRODUCTION ANALYSIS DURING FERMENTATION IN VITRO

Lactobacilli and Bifidobacteria ferment carbohydrates through a pathway mediated by the glycolytic enzymes in which the main end products are SCFA (Grootaert et al., 2007). Butyrate, Propionate and Acetate are the major SCFA produced during fermentation of carbohydrates in the large bowel (Maniserri C et al, 2009).

A study on bioactive xylooligosaccharides from wheat bran soluble polysaccharides reported that Acetate was the chief SCFA liberated due to in vitro fermentation of xylooligosaccharides (Maniserri C et al, 2009).

Another study on prebiotic effects of Xylooligosaccharides on the improvement of microbiota balance in human subjects reported that the abundance of pathogenic bacteria, *Clostridium perfringens*, was significantly lower in the fecal samples of the XOS group than in those of the control group. This was explained by the XOS suppressing the growth of *Clostridium perfringens*; the mechanisms underlying this effect were likely due to the production of short-chain fatty acids (SCFAs) via the fermentation of XOS in the colon. A decrease in intestinal pH has been reported as a consequence of the increased SCFA production which subsequently inhibits the overgrowth of pathogenic bacteria (Lin SH et al, 2016).

A comparative study of synbiotic and prebiotic supplementation on gut health, SCFA, hs-CRP and lipid profile of type 2 diabetic subjects with pre hypertension concluded that daily intake of 1 g synbiotic product and 10 ml FOS improved gut health, hs-CRP, lipid profile and short chain fatty acids (SCFA) of the subjects which may be due to increased production of SCFA (Sheth M et al, 2015).

Another study on consumption of XOS in combination with inulin did not decrease the concentrations of acetate and *p*-cresol, but increased the faecal concentrations of total SCFA and propionate (Lecerf JM et al, 2012).

In this study as shown in fig 3.4.1, 3.4.2 and 3.4.3, Acetate was produced the most followed by Propionate and Butyrate. *Bifidobacterium adolescentis* produced (331%) more of Butyrate and Propionate respectively on its exposure to XOS ($p \leq 0.01$), whereas, *Lactobacillus plantarum* produced more acetate as compared to *Bifidobacterium adolescentis* ($p \leq 0.001$). Production of all the three SCFA reduced (20%-48%) in case of *Escherichia coli* on its exposure to XOS ($p \leq 0.001$).

Fig. 3.4.1. Production of Propionate by *Lactobacillus plantarum, Bifidobacterium adolescentis* and *Escherichia coli*

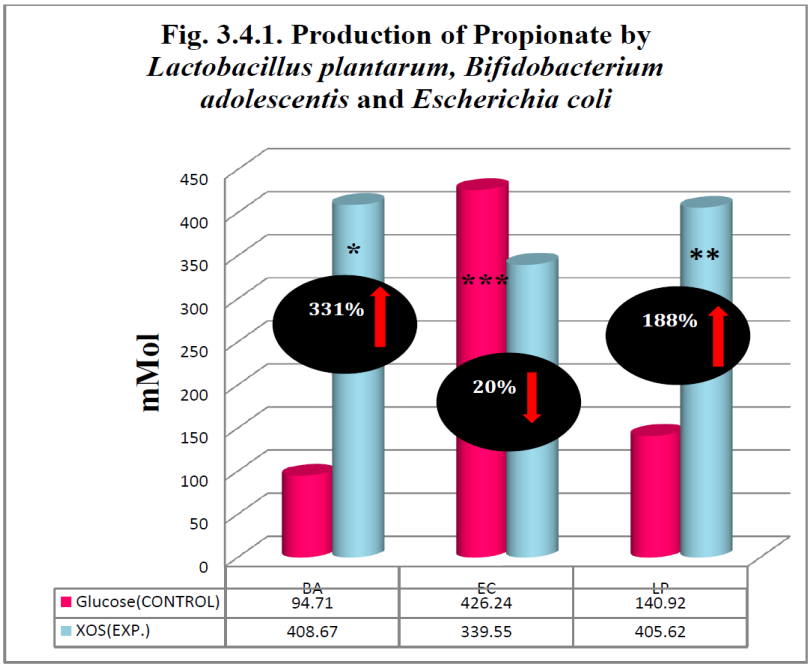


Fig. 3.4.2. Production of Butyrate by *Lactobacillus plantarum, Bifidobacterium adolescentis* and *Escherichia coli*

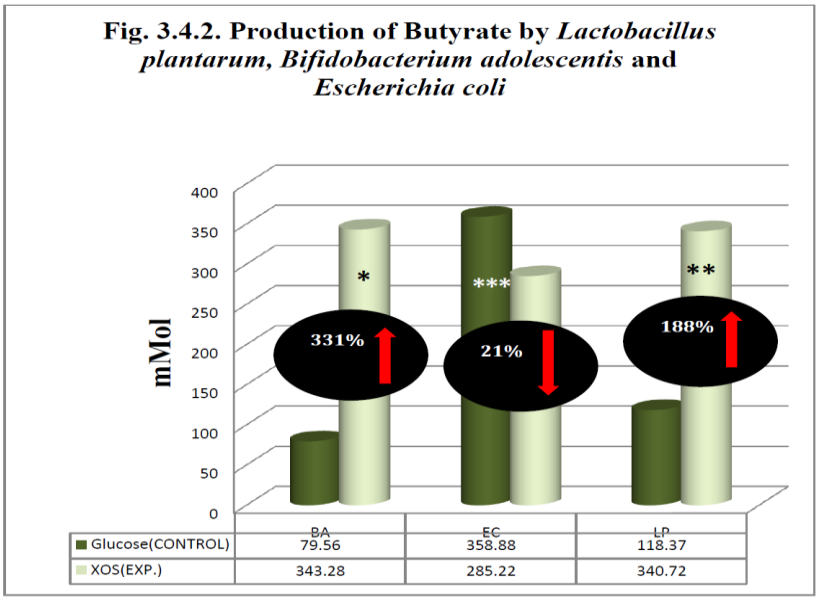
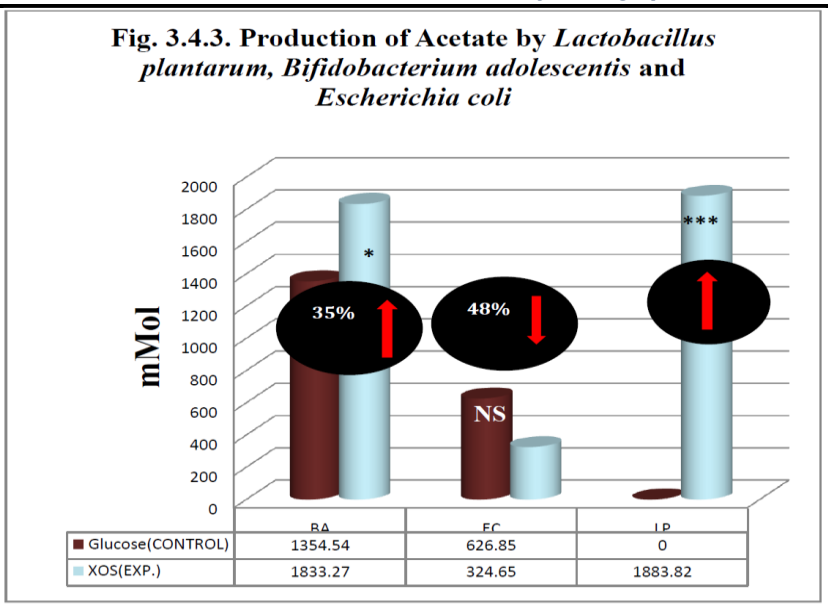


Fig. 3.4.3. Production of Acetate by *Lactobacillus plantarum, Bifidobacterium adolescentis* and *Escherichia coli*



Significant at *p≤0.05, **p≤0.01, *** p≤0.001

4. CONCLUSION

This study has successfully established the prebiotic potential of XOS in terms of acid tolerance, bile resistance, growth of probiotic bacteria and production of SCFA. Limited researches have been conducted on prebiotic XOS. Therefore, for discussion references were taken with regards to other prebiotics such as FOS, inulin etc. This study will help to create a strong evidence based data to prove the prebiotic potential of XOS. Further studies can be undertaken to demonstrate the clinical efficacy of XOS intake with respect to various non communicable diseases.

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