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Analyzing the Prebiotic Potential of Glucosamine for Targeting the Gut Microbiome Health

Pranav Pancham[1,2], Divya Jindal[1], Archita Gupta[3,4], Deepshikha Yadav[5,6,7], Shriya Agarwal[8], Saurabh Jha[9], Manisha Singh[1*]

[1]Department of Biotechnology, Jaypee Institute of Information Technology (JIIT), Noida, India
[2]Neuroimmunology Laboratory, School of Medicine, Western Sydney University, Campbelltown, Australia
[3]Birla Institute of Technology, Mesra, India
[4]Indian Institute of Technology-Kanpur, Kanpur, India
[5]Bhartiya Nirdeshak Dravya Division, CSIR-National Physical Laboratory, New Delhi, India
[6]Physico-Mechanical Metrology Division, CSIR-National Physical Laboratory, New Delhi, India
[7]Academy of Scientific and Innovation Research (AcSIR), Ghaziabad, India
[8]School of Natural Sciences, Macquarie University, Sydney, Australia
[9]Department of Biotechnology, School of Engineering and Technology, Sharda University, Greater Noida, India
Email: pranavpanchm@gmail.com, divyajindal065@gmail.com, *manishasingh1295@gmail.com, pranavpanchm@gmail.com, architagupta6@gmail.com, deepshikha.yadav93@gmail.com, shriyaz.dd@gmail.com, saurabh.jha@sharda.ac.in

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Abstract

Recognizing the composition and modulation of the microbiome, a viable therapeutic tool for multi-targeted therapy is a new strategy that has recently been explored. Glucosamine (GS) is being studied for its prebiotic potential in addition to being the most abundant and naturally occurring amino monosaccharide. The current study focuses on glucosamine's prebiotic potential by assessing the stability of various GS concentrations (1% - 5%) in the gastrointestinal tract (GIT) and its ability to be fermented by the gut microbiota. The results showed that GS stimulated the most growth in L. acidophilus even after a longer incubation time than B. bifidum and L. acidophilus growth was concentration-dependent, with maximum growth at 3% with a simultaneous decrease in pH (5.6 - 1.7). The decrease in GS concentration with time also represented the growth of bacterial species, demonstrating the species' utilization of GS. Furthermore, at 3%, GS also represented the prebiotic index of 1.9. In addition, the concentration of GS in various simulated GIT fluids was estimated in both fast and fed conditions to examine GS stability at various levels in the gut. The results showed that GS remained unaffected and non-digestible in all of the simulated GIT fluids (salivary, gastric, intestinal, and colonic), but there was a slight decrease in GS concentration (2.8%) in the fasted state of gastric fluid due to low pH levels (1.6). As a result, the findings are conclusive and suggest that GS possesses prebiotic properties.

Keywords

Microbiome, Enteric Nervous System (ENS), Prebiotic Index, Hexosamine Biosynthetic Pathway (HBP), Vagal Afferents, Phosphotransferase System

1. Introduction

Around 10,000 different species of non-pathogenic bacteria make up the human microbiome and their symbiotic connections with the host are crucial for their digestive, respiratory, hepatic, and immunological functions [1]. As a result, in today's time, supplements and nutraceuticals containing prebiotics, probiotics, and synbiotics fit the criteria for such a holistic treatment strategy, as do novel drugs. Moreover, it has been established that a probiotic-induced regulated microbiome can alleviate various types of neurological disorders too, through multiple pathways. The microbiome-gut-brain axis has been known to produce significant evidence for its two-way connectivity between brain and gut microflora, thus having a major effect on gastrointestinal health and inciting enteric and central nervous systems (CNS) signaling pathways. Some of the consequences include antioxidant production and subsequent neutralization of reactive oxygen species (ROS) in the gastrointestinal (GI) tract. Furthermore, they also help in the downregulation of cytokine-induced proinflammatory cascades and enhancing epithelial barrier functions to protect the enteric nervous system (ENS) from potentially toxic compounds [2]. The microbiome can communicate with the CNS and ENS in a bilateral communication channel that makes up the microbiome-gut-brain axis even more important for the efficiency and success of the proposed multi-targeted therapeutic approach combining prebiotics, probiotics, and synbiotics. Similarly, the health benefits of prebiotic chemicals have been extensively researched in the development of nutraceuticals, medicines, and functional foods further leading to the development of various natural substances that have been studied for their overall prebiotic potential and intestinal stability [3]. One such potential compound, Glucosamine (GS), a naturally occurring amino sugar, extracted from crustacean chitin is normally produced in the human body through cell glucose metabolism and has shown promising results [4].

GS is known to exhibit reduced chronic inflammation in arthritis and aids in the prevention of osteoporosis progression after menopause. Simultaneously, it's also a frequent element of brain glycogen supply and GS storage is a support for a range of glycoconjugates present, according to the latest report [5]. Owing to its capacity to pass through the blood-brain barrier (BBB), GS accounts for 25% of brain glycogen's covalently bonded sugar monomers and gets accumulated in synaptosomes and ganglions, resulting in the production of gangliosides and glycoproteins that are necessary for brain function. Also, N-linked glycosylation gets involved in numerous cognitive abilities, involving synaptic plasticity, neu-

rite outgrowth, and neuron shape, as well as learning and memory formation, thus GS balance in the gut may have a favorable effect on the cognitive performance of the CCNS-compromised patients [6]. Now, we can correlate the ability of GS as a prebiotic for multi-targeted therapeutic strategy along with pharmaceutical techniques in neural illnesses because of its favorable impacts on the gut microbiome and brain. Many studies suggest that GS may have potential as a nutraceutical and novel biological prophylactic in the treatment of neurodegenerative diseases (NDDs) due to their anti-inflammatory and antioxidant properties, ability to improve cognitive and metabolic activity, and capacity to produce essential metabolites for gut and brain barrier permeability [7] [8]. Subsequently, recent research has focused on the role of GS in the hexosamine biosynthetic pathway (HBP). In the HBP pathway glucose is speedily phosphorylated to glucose-6-P inside the cells, which then is transformed to fructose-6-P and joins the glycolysis process to generate energy and CO_2. Glucose-6-P can also be bio-transformed into glucose-1-P, which is then used in the glycogenesis process to generate glycogen. A tiny amount of fructose 6 phosphate (about 3%) enters the HBP, where glutamine fructose 6 phosphate amidotransferase converts fructose 6 phosphates to Glucosamine 6 phosphate (GlcN-6-P) (GFAT) [9]. Nevertheless, some studies highlight a potential mode of action linked to the probiotic benefits of GS in a variety of bacterial species wherein the fructose-6-phosphate phosphor ketolase (F6PPK) pathway, is also recognized as the "bifid shunt". It is a central metabolic pathway in which the hypothesized GS absorption takes place through the use of a phosphotransferase system (PTS) and via probable transformation and breakdown of Glucosamine hydrochloride to Glucosamine (GlcN) and thus, further conversion to N-acetylglucosamine (GlcNAc) [10] [11]. Also, L. acidophilus includes a route for converting N-acetylglucosamine to fructose 6 phosphate, which can then be used in the homofermentative metabolic pathway to create secondary metabolites [12] [13]. On the other hand, short-chain fatty acids (SCFAs) have exhibited promising biotherapeutic capabilities by improving the barrier function of the gut epithelium, modulating gut microbiota, and immunomodulation, highlighting the end outcomes of the bifid shunt [14] [15]. At the intersection of the gut, there's the presence of chemo-sensing interconnections, involving gut enteroendocrine cells (EECs), and vagal afferents, they sense microbiota signals via toll-like receptors (TLRs) (**Figure 1**). This further recognizes the bacterial products including lipopolysaccharides (LPS) and others expressed by EECs, as well as receptors for microbiota compounds like SCFAs.

Moreover, it is been also observed that EECs communicate with vagal afferents either directly by releasing serotonin (5-hydroxytryptamine, 5-HT), which activates 5-HT3 receptors on vagal afferent fibers, or indirectly through gut hormones (cholecystokinin-CCK), glucagon-like peptide-1, and peptide YY. They all target the brain via vagal afferents that are responsive to both mechanical and chemical stimuli. In this way, alterations in the microbiota can have an

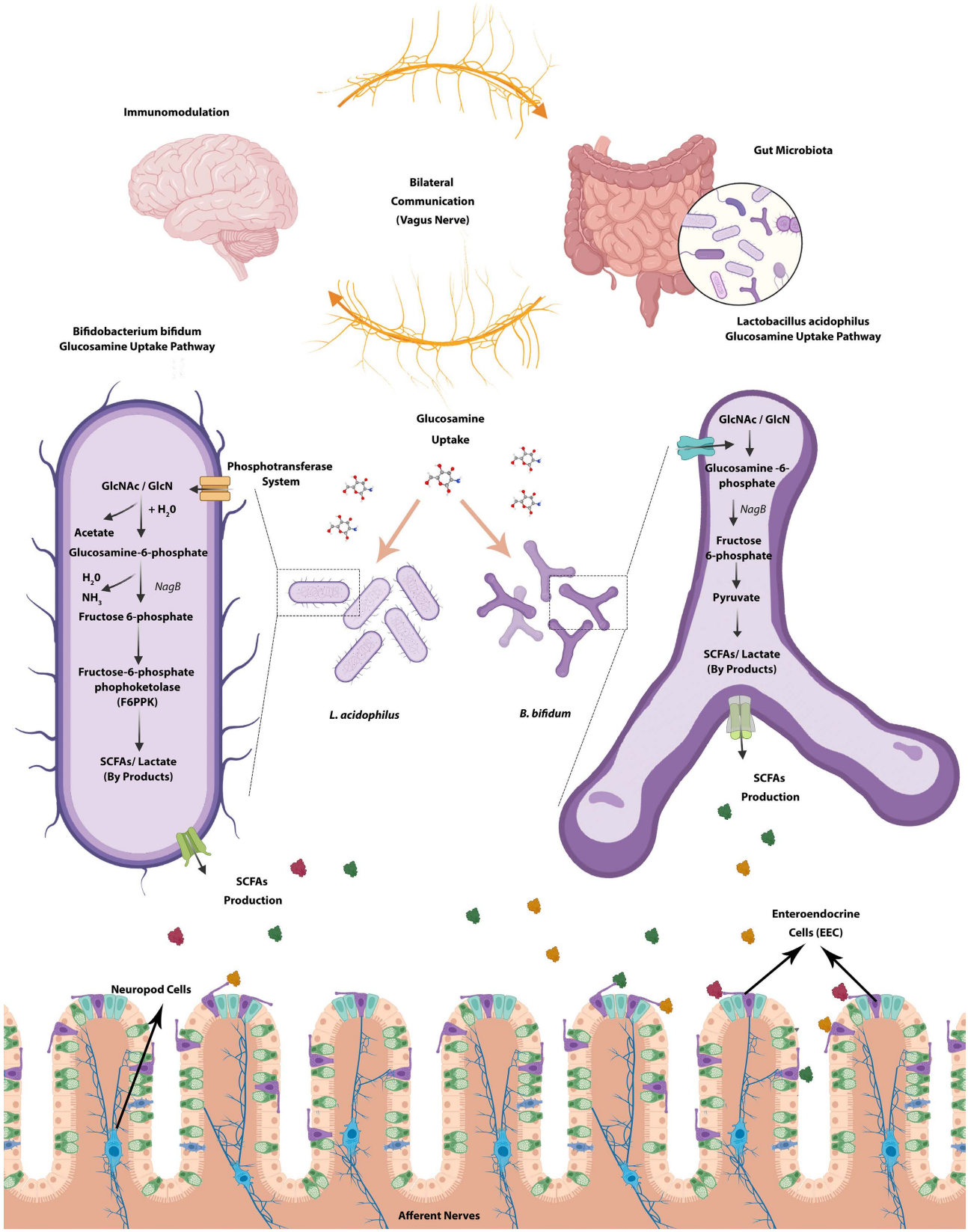

Figure 1. Schematic representation of GS utilization in gut microbiome along with its effect on L. acidophilus and B. bifidum. Abbreviations: SCFAs: Short chain fatty acids; EEC: Enteroendocrine cells.

impact on CNS cells, notably astrocytes and microglia, causing them to change their roles [16] [17].

Besides this, GS is also reported to exhibit the fermentation property for many essential bacterial species such as Lactobacilli strains, L. plantarum, L. casei, L. leichmanii), Staphylococcus, Streptococcus, Leuconostoc, Proteus vulgaris, and Enterococcus faecalis present in the gut. In addition to this, the mucosal cells in GIT synthesize and secrete protective mucin throughout the GIT by utilizing GS. It also has limiting induction of operons and differential activity of allosteric enzymes thus, protecting and reducing the growth of pathogenic bacteria and further, can be used as a natural prebiotic in various functional foods. The present study aims to explore the possibility of GS being a potential prebiotic option.

2. Materials and Methods

2.1. Materials Required

The bacterial strains Lactobacillus acidophilus (MTCC 10307), Bifidobacterium bifidum (MTCC 5398), and Clostridium deficile spp. (ATCC 1382), were procured from the Institute of Microbial Technology (IMTECH), Chandigarh, Punjab, India. DNS (Dinitro salicylic acid) reagent, MRS (de Man, Rogosa, and Sharpe) media, Potassium phosphate monobasic, sodium phosphate dibasic, and maleic acid were obtained from Sigma Aldrich, the USA, and LB (Luria-Bertani) media from CDH, New Delhi, India. Glucosamine hydrochloride was procured from SRL, New Delhi, India. Lecithin and pepsin were acquired from HI media, Mumbai, India. All other chemicals used were of analytical grade.

2.2. Quantification of GS Degradation and Bacterial Growth

Since, GIT microbiota genera and species (Lactobacilli, Streptococcus, Staphylococcus, Leuconostoc strains, Escherichia coli, Enterococcus faecalis, Proteus vulgaris, and Bacillus coli) are known to ferment many sugars like lactose, fructooligosaccharides, galactooligosaccharides, chitosan oligosaccharide, etc. These sugars are utilized by GIT microorganisms to compose more peptidoglycan and lipopolysaccharide contents in the cell wall, which further promotes bacterial cell division [18] [19]. Now, Glucosamine (GS), being a monosaccharide unit has certain GIT functioning as it is being reported to get utilized by mucosal cells to secrete mucin in GIT. This possibly qualifies GS as a prebiotic substance [20]. The total GS concentration available in the solution was quantitatively measured by the dinitro salicylic acid (DNS) assay. Here, under alkaline conditions, DNS reacts with the free carbonyl group of the reducing sugar (GS) leading to the formation of an aromatic compound 3-amino-5-nitro salicylic acid. For this, 1 ml test sample solution in different concentrations (0.2 - 1 mg/ml GS) was taken and the volume was made up to 3 ml. To this 3 ml DNS, the reagent was added and incubated at 100˚C (boiling water bath) for 5 minutes and detected at 540 nm [21].

2.3. Microbial Growth and Culturing

The beneficial microbes selected for the present study were L. acidophilus and B. bifidum. They were cultivated by inoculating 100 µl of stock in 50 ml MRS medium. While C. deficile was taken as pathogenic control for prebiotic activity and was propagated in Clostridial Agar (Himedia). L. acidophilus and C. deficile were grown aerobically at 37˚C for 24 h and B. bifidum was grown anaerobically in a desiccator at 37˚C for 24 h [22] [23]. The medium was supplemented with 5% FOS as positive control and GS as a test sample with different concentrations (1% - 5%). After this, to evaluate the prebiotic activity of GS, samples were withdrawn at different time intervals (0, 12, 24, 36, and 48 h).

2.4. Bacterial Growth Enumeration and GS Metabolism

The change in optical density is considered a reflecting factor for the growth of bacteria therefore, the growth of bacteria (L. acidophilus, C. deficile, and B. bifidum) in the presence of different GS concentrations (1% - 5%) was recorded concerning varying time intervals (0, 12, 24, 36, 48 h) at 600 nm (L. acidophilus and C. deficile) [24] and 660 nm (B. bifidum) [25] respectively. Similarly, to ensure the prebiotic activity of GS, the fermentation of GS by the mentioned microbes was also analyzed by the DNS method. Also, the gram staining was done parallel, to rule out the optical density imminent due to any contamination.

2.5. pH Index Monitoring

It is noted that, if there is any change in the growth of the bacteria, the pH of the medium varies significantly. Moreover, the metabolites formed during the growth of bacteria could also affect the pH of the medium [26]. Hence, considering the fact, the pH of the fermented medium with different GS concentrations (1% - 5%) and time intervals (0, 12, 24, 36, 48 h) was measured.

2.6. Measuring Prebiotic Index (PI)

The prebiotic activity is selective having a direct relation with the growth of beneficial bacteria and the opposite relation with that of enteric bacteria. To compare the prebiotic activity of GS within its different concentrations (1% - 5%) and in comparison, with the positive control (FOS), the prebiotic index is calculated. This can be expressed as:

$$\text{Bacterial count}\left(BC\right)=\frac{B\text{ sample}}{B\text{ inoculation}} \tag{1}$$

where B represents the number of bacteria

$$\text{Prebiotic index}=\frac{\Sigma BCb-\Sigma BCe}{BCt} \tag{2}$$

where, BCb, BCe and BCt represent the bacterial count for beneficial, enteric, and total bacteria respectively [27].

2.7. *In-Vitro* Digestion Studies for GS

The stability testing of any product to be claimed as prebiotic is done to ensure non-degradation, non-digestibility, and resistance for gastric acidity of the compound in various digestive fluids [28]. To study the digestion of GS under acidic conditions faced in the gastrointestinal tract, different simulated fluids starting from the first exposure by salivary fluid in the mouth, gastric, intestinal, and colonic fluids were prepared. Then the estimation of GS content in each simulated fluid was analyzed by DNS assay. Here fructooligosaccharide (FOS) was taken as a positive control/standard [29] [30].

2.8. Digestion of GS in Simulated Salivary Fluid (SSF)

At first, the stability of GS was tested in simulated salivary fluid (SSF) which is imitated by adding (all components in mg/ml): 0.72 potassium chloride, 0.22 calcium chloride dihydrate, 0.6 sodium chloride, 0.68 potassium phosphate monobasic, 0.866 sodium phosphate dibasic, 1.5 potassium bicarbonate, 0.06 potassium thiocyanate and 0.03 citric acid [31]. The pH of the SSF was maintained at 6.5 [32]. Now, 5 ml of test solution (5% GS) was added to an equivalent amount of SSF and incubated at room temperature for 30 minutes. As residence time for mechanical digestion in the mouth is reported to be a maximum of 15 - 30 minutes. Meanwhile, 1 ml of the reaction mixture was withdrawn after every 5 minutes for DNS estimation of GS, and to maintain equilibrium equal volume (1 ml) of SSF was compensated back.

2.9. Digestion of GS in Simulated Gastric Fluid (SGF)

The non-digestibility of GS was again analyzed in the simulated gastric fluid under two conditions fast (pH 1.6) and fed (pH 5) states. The fasted state simulated gastric fluid (FaSSGF) was prepared using sodium taurocholate (80 uM), lecithin (20 μM), pepsin (0.1 mg/ml), and sodium chloride (34.2 mM) whereas, the fed state simulated gastric fluid (FeSSGF) was prepared using sodium chloride (237.02 mM), acetic acid (17.12 mM), sodium acetate (29.75 mM) and milk/buffer (1:1, v/v) [33] [34]. The process of digestion is completed in different stages and after the cephalic stage of secretion, and stimulation comes the digestive phase which takes around 2 - 3 hours to further process the ingested compound [35]. Hence, here in the experiment, the test sample (5% GS) was incubated in the above-mentioned SGF for 3 hours. Meanwhile, 1 ml of the test sample was withdrawn for DNS estimation of GS every 30 mins and the same amount is compensated back.

2.10. Digestion of GS in Simulated Intestinal Fluid (SIF)

Following the digestion by gastric fluids, comes the intestinal phase where pH varies from 6.5 (fasted state) to 5 (fed state). The composition of simulated fluid for fasted (FaSSIF) and fed state (FeSSIF) of the intestine remains almost the same except for the concentration of chemical compounds which is reported as

lecithin (0.2 mM - FaSSIF, 2 mM - FeSSIF), maleic acid (19.12 mM - FaSSIF, 44 mM - FeSSIF), sodium hydroxide (34.8 mM - FaSSIF, 65.3 mM - FeSSIF) and sodium chloride (68.62 mM - FaSSIF, 122.8 mM - FeSSIF) [33]. The test sample was incubated with both fluids (FaSSIF and FeSSIF) for 2 hours as that is the maximum residence time of any compound in the intestine and as mentioned before the samples were withdrawn for further analysis.

2.11. Digestion of GS in Simulated Colonic Fluid (SCoF)

Lastly, food digestion gets completed as it passes through the colonic conditions. The simulated fluids for the initial part of the colon *i.e.*, ascending colon (SCoF1) is prepared (in mg/ml) by adding 0.2 potassium chloride, 8 sodium chloride, 0.24 potassium phosphate monobasic, and 1.44 sodium phosphate dibasic and the pH at 7. Whereas, the later part of the colon-descending colon (SCoF2) is simulated by using (in mM) 170 acetic acid and 157 sodium hydroxides, at pH 5.8 [36]. The colon or large intestine area of the digestive system absorbs and retains water, and electrolytes along with waste material from undigested food and takes around 7 - 8 hours to complete this process [37]. The test samples were incubated for 8 hours and analyzed by taking the samples as described before.

Therefore, for any compound to be considered prebiotic it must be stable and not get digested or hydrolyzed through the GIT and persist in a colonic phase of digestion so that it remains available for stimulating the residence of gut microflora.

2.12. Statistical Analysis

The two-way ANOVA test was used to test all the obtained data that presented as mean ± standard deviation and it was found that at $p < 0.01$ the values were deemed significant.

3. Results and Discussion

3.1. Quantification of GS Degradation and Bacterial Growth

Bacterial Growth Enumeration

To study the effect of GS on the growth of the beneficial bacteria, the medium was supplemented with GS and FOS (1% - 5%) at appropriate growth conditions up to duration of 48 hours. Belorkar SA *et al.*, 2016 reported that FOS remains available for fermentation by gut microflora and can increase bacterial biomass thus stimulating the growth of L. acidophilus and B. bifidum. GS stimulated the growth of L. acidophilus more than that of B. bifidum.

GS was found to have a more potential growth stimulatory effect on L. acidophilus than B. bifidum. The bacterial cell growth was found to be concentration and time-dependent and maximum growth was seen at 3% (**Figure 2**). The prominent growth of L. acidophilus can be observed at 3% at different time intervals (**Figure 3**). In the case of B. bifidum, however, the growth increased with

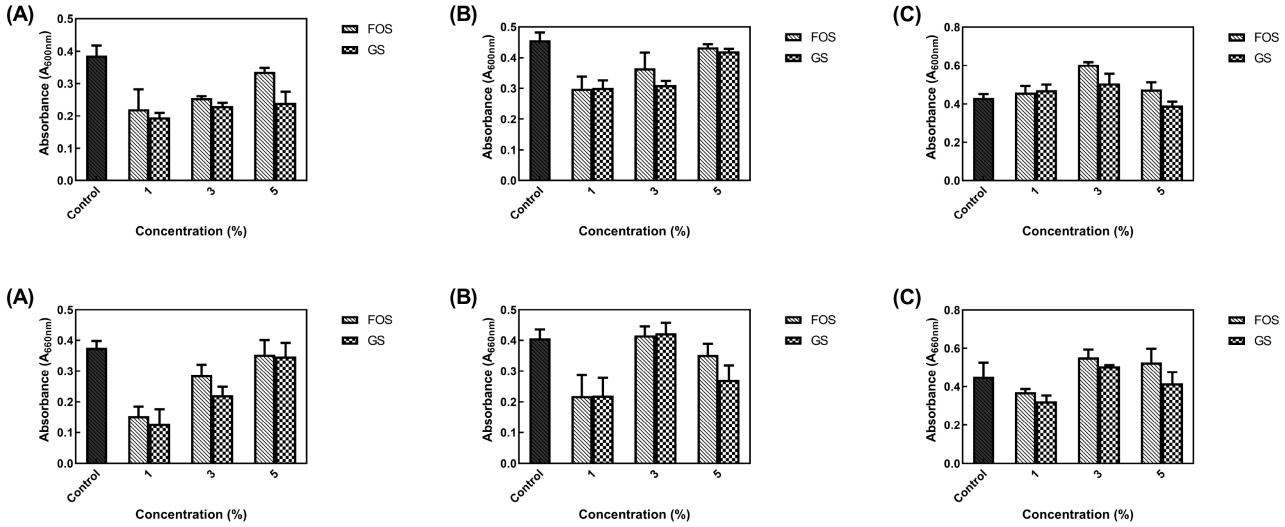

Figure 2. Graph representing the growth of L. acidophilus (Top) and B. bifidum (Bottom) at different GS and FOS concentrations after (A) 12 hr (B) 24 hr (C) 48 hr. Abbreviation: B. bifidum = Bifidobacterium bifidum, FOS = Fructo-oligosaccharide GS = Glucosamine, L. acidophilus = Lactobacillus acidophilus.

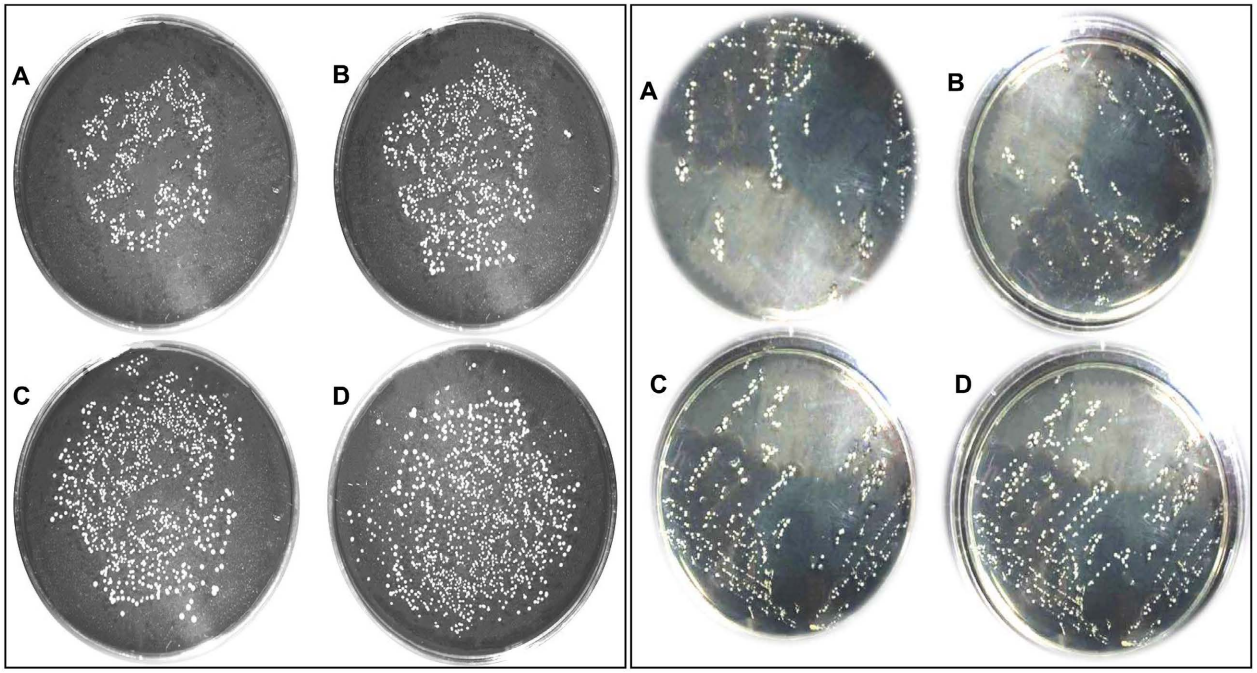

Figure 3. Image representing L. acidophilus (Left) and B. bifidum (Right) growth under (A) control after 48 hr and 3% GS after (B) 12 hr (C) 24 hr (D) 48 hr on MRS agar. Abbreviation: B. bifidum = Bifidobacterium bifidum, L. acidophilus = Lactobacillus acidophilus, MRS = de Man, Rogosa, and Sharpe.

an increase in the concentration of GS or FOS, there was a decrease in the growth after 24 hours for GS concentration above 3% (**Figure 2**) indicating maximum growth at that particular concentration but not as prominent as that of L. acidophilus. The growth of B. bifidum can be observed with maximum growth at 3% for different incubation times (**Figure 3**).

The effect of GS was also studied on the growth of an enteric bacterium, C.

deficile. The results showed that there was a tremendous increase in the growth of the bacterium in the basal medium (LB). It was reported earlier that the growth of pathogenic organisms was inhibited in the presence of FOS, so after the addition of GS and FOS in the basal medium of C. deficile, the growth was not significantly increased indicating that they are not stimulating the growth of the enteric bacterium and maintained after 36 hours of GS or FOS supplementation (Data not represented). Moreover, slight depletion in growth was observed in both 5% GS and FOS.

The results indicate that GS can potentially stimulate the growth of the probiotic species and also helps in maintaining the growth of the enteric bacterium.

3.2. GS Metabolism

The growth of the bacteria in the presence of GS was also evaluated by measuring the amount of GS fermented by them. To study the same, reducing sugar content remaining after fermentation was measured by the analytical method. It was observed that GS or FOS was utilized by the bacteria as its concentration in the medium decreased with time. Barrangou *et al.*, 2003 reported that bacteria withdraw sugars from the environment for their growth presenting similar results [38].

The decrease in GS concentration was more prominent in fermentation by B. bifidum (2.73%) (**Figure 4**) as compared to L. acidophilus (3.11%) when supplemented with 5% GS (**Figure 4**). The results suggest that GS (0.6% - 3.11%) is utilized equivalent to that of FOS (0.59% - 1.95%) by L. acidophilus with each concentration supplemented (1% - 5%).

In the case of C. deficile, with increasing concentration of supplements, the fermentation of compounds increased and was more significant when supplemented with 3% FOS. However, the GS (1.6%) was not metabolized much as compared to FOS (0.7%) when supplemented with 3% of GS or FOS. The concentration of GS was also maintained and no further decrease was observed after 36 hours of fermentation. The results show that FOS was utilized more than GS for the growth of C. deficile.

3.3. Monitoring pH Index

Bacterial division over time leads to the formation of compounds such as acetate and lactic acid which could lower the pH of the medium as exhibited by Macfarlane and Gibson, 1997. A decrease in pH is an indicative parameter of bacterial growth. In the case of L. acidophilus, the pH decrease (2.8 - 0.7) was observed with an increasing concentration of GS (1% - 5%) (**Figure 5**). However, the fall in pH was also concentration-dependent during B. bifidum growth but more decrease in pH (3) was observed with 5% FOS than pH (3.6) with 5% GS after 48 hours (**Figure 5**). As the growth of C. deficile increases, the pH should remain neutral as that of the basal medium. However, there was a slight decrease in pH observed after supplementation of FOS or GS. But after a longer duration of exposure, the change was not significant indicating that the growth of C. deficile

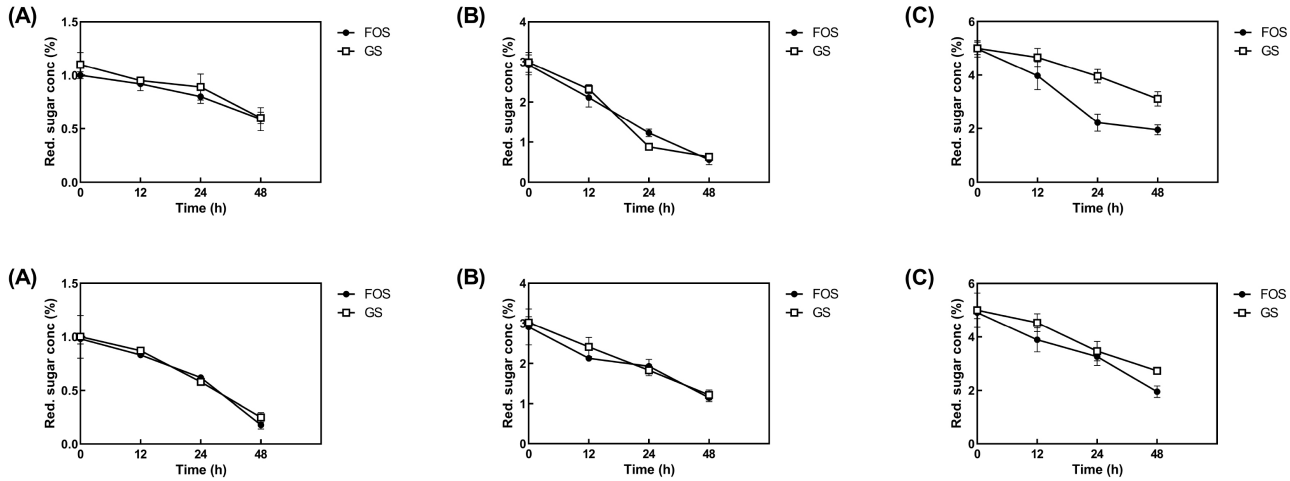

Figure 4. GS and FOS metabolized by L. acidophilus (Top) and B. bifidum (Bottom) after (A) 12 h (B) 24 h (C) 48 h. Abbreviation: B. bifidum = Bifidobacterium bifidum, FOS = Fructo-oligosaccharide GS = Glucosamine, L. acidophilus = Lactobacillus acidophilus.

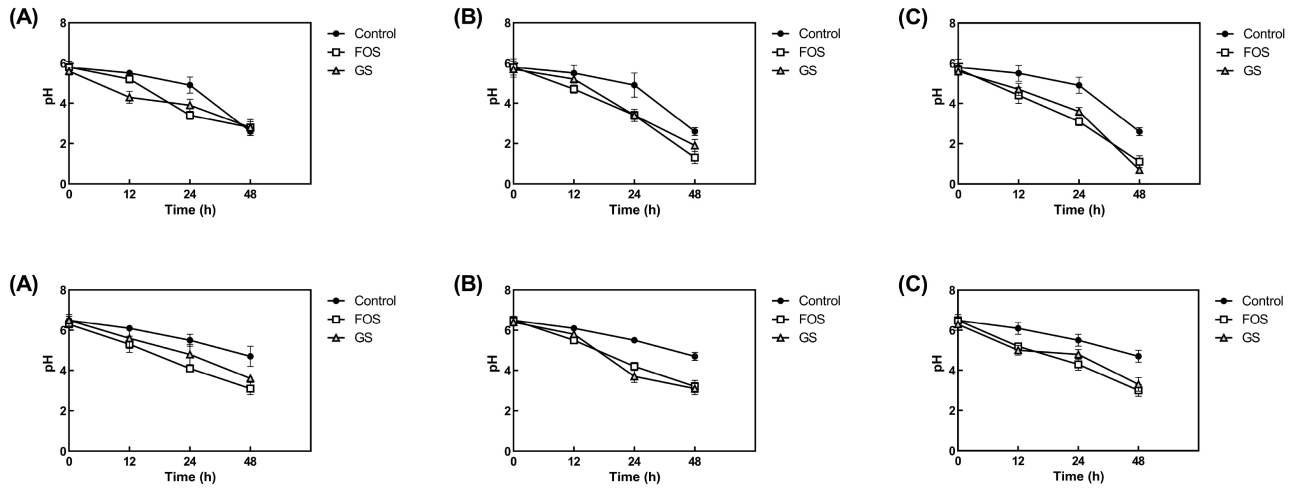

Figure 5. pH variation under L. acidophilus (Top) and B. bifidum (Bottom) growth after (A) 12 hr (B) 24 hr (C) 48 hr. Abbreviation: B. bifidum = Bifidobacterium bifidum, L. acidophilus = Lactobacillus acidophilus.

was not stimulated by them but rather maintained the same after 36 hours with no change in pH.

3.4. Quantitative Approach-Prebiotic Index (PI)

To get the general measure of prebiotic activity, a quantitative approach was used by calculating PI for different concentrations of GS and FOS. PI represents the relationship between the growth of beneficial bacteria and that of non-beneficial bacteria with respect to the total bacteria.

For both FOS and GS, PI values increased with concentration and time. However, PI for FOS was observed more than that of GS. FOS showed the highest PI of 2.8 whereas GS showed the highest PI of 1.9 at 3% after 48 hours (**Figure 6**). In the comparative study, similar prebiotic activity for FOS was observed. The results suggested that GS has a potential prebiotic effect although less than that of FOS.

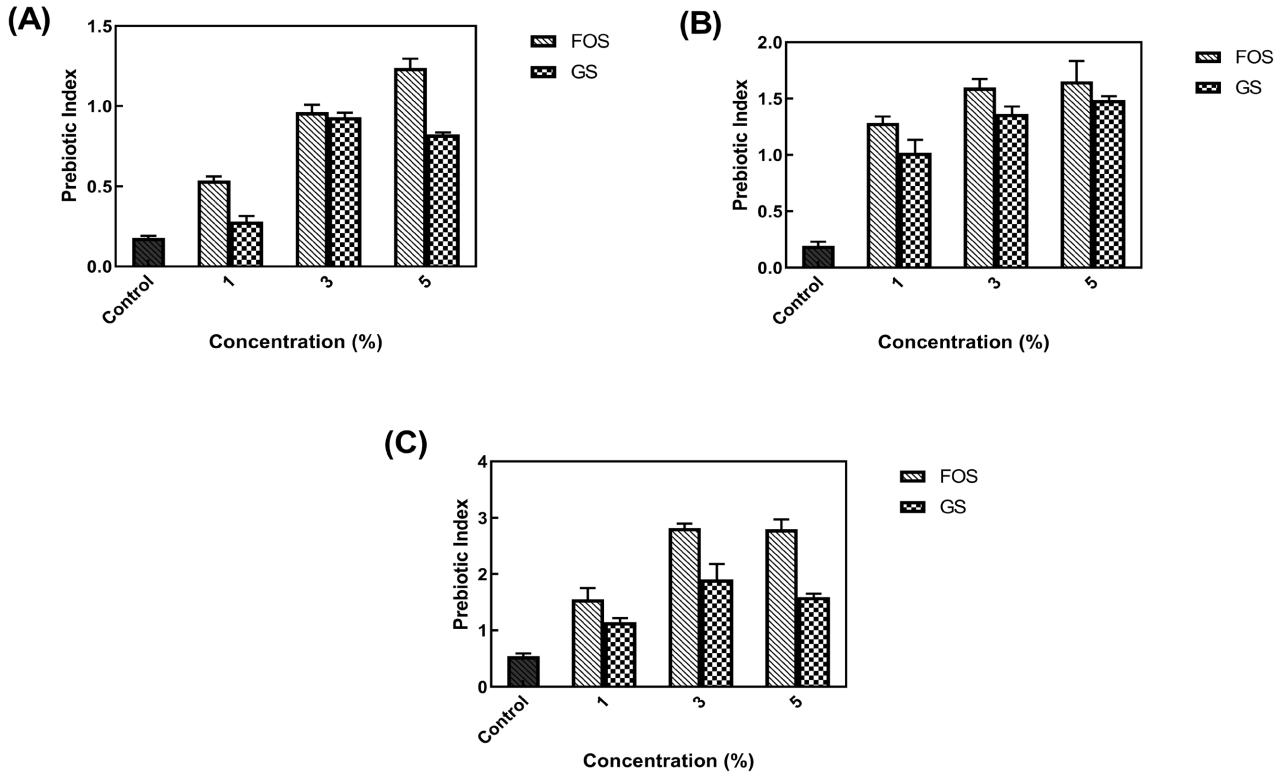

Figure 6. Prebiotic index of GS and FOS after (A) 12 h (B) 24 h (C) 48 h. Abbreviation: FOS = Fructo-oligosaccharide, GS = Glucosamine.

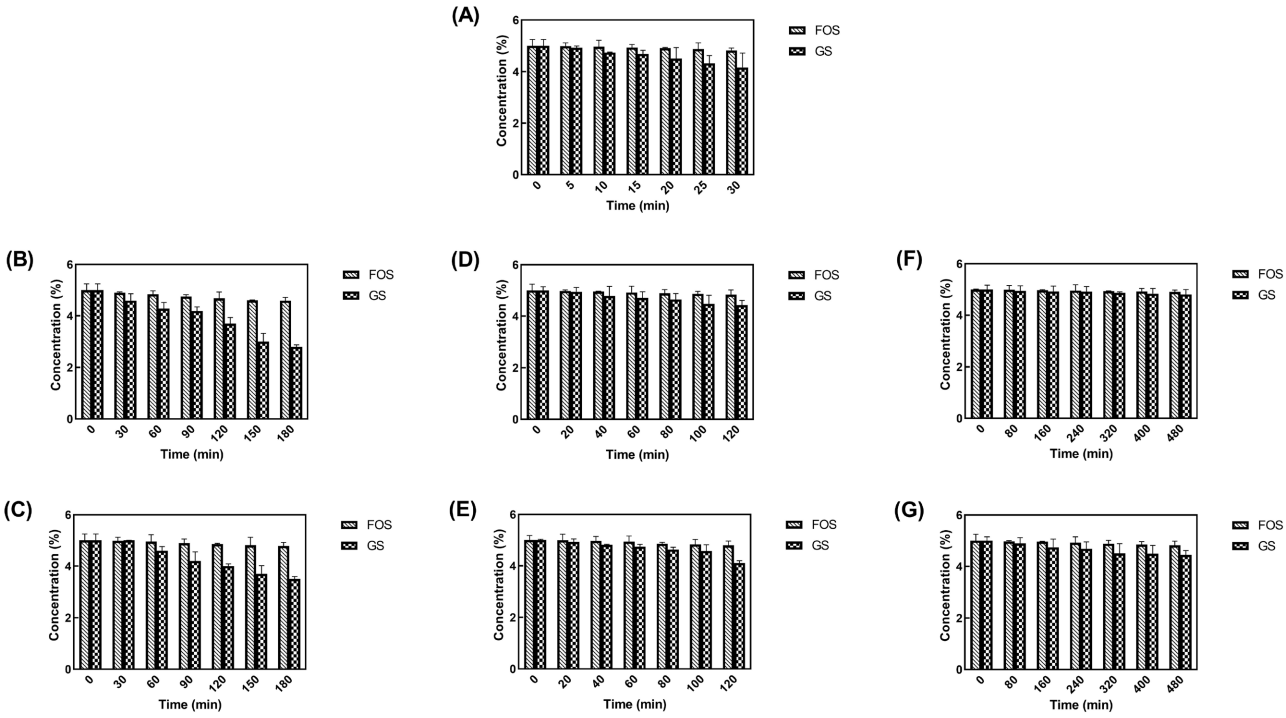

Figure 7. GS digestion in various simulated fluids (A) SSF (B) FaSSGF (C) FeSSGF (D) FaSSIF (E) FeSSIF (F) SCoF1 (G) SCoF2. Abbreviation: FaSSGF = Fasted State Simulated Gastric Fluid, FeSSGF = Fed State Simulated Gastric Fluid, FaSSIF = Fasted State Simulated Intestinal Fluid, FeSSIF = Fed State Simulated Intestinal Fluid, GS = Glucosamine, SCoF1 = Simulated Ascending Colonic Fluid, SCoF2 = Simulated Descending Colonic Fluid, SSF = Simulated Salivary Fluid.

3.5. *In-Vitro* Digestion Studies for GS

The digestion of GS was studied in different simulated fluids to ensure that non-digested GS reaches the colon where it can remain available for the growth of beneficial bacteria. In this study, it was observed that GS was getting more digested by the simulated fluids as compared to FOS since the acidic conditions in GIT resist FOS to be hydrolyzed to monosaccharides. It was observed that in simulated salivary fluid, GS was digested and remained at 4.16%. While GS was found more susceptible towards the pH of the gastric fluid in a fasted state (1.6) leading to its maximum digestion of up to 2.8% (**Figure 7**). While in the case of simulated intestinal fluid digestibility was observed to be 4.44% and 4.11% in fasted and fed states respectively after 48 hours. Moreover, the ascending and descending colonic conditions were studied where digestibility of 4.8% was observed in ascending colonic condition and 4.45% in descending colonic condition.

4. Conclusion

The amount of growth of L. acidophilus increased with increasing concentration of GS with respect to time up to 24 hours. After 48 hours maximum growth was observed in MRS broth supplemented with 3% GS, simultaneously the results were supported by the amount of GS metabolized and the change in pH due to bacterial growth. The study suggested that there was a decrease in the growth of B. bifidum after 24 hours of GS supplementation of more than 3%. The growth of L. acidophilus was observed to be more remarkable than B. bifidum. The study of the prebiotic index of GS showed that better prebiotic activity is represented by supplementation of 3% GS for a longer duration of time but the results were slightly lower as compared to FOS. The GS was also found to be stable under acidic conditions of various GIT-simulated fluids exhibiting only a reduction of GS concentration to 2.8% from 5% in the gastric simulated fluid. The present study provides ancillary results showing the growth of prominent probiotic bacteria and the metabolism of GS in presence of them along with the non-digestibility of GS in acidic conditions of GIT suggesting GS to have potential use as a prebiotic functional food.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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