# Determination of the optimum growth conditions for three Lactobacillus species in culture media prepared using Fructooligosaccharide

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**Abstract**
This laboratory study was conducted to determine the optimum conditions for the growth of Lactobacillus casei, L. paracasei, and L. plantarum bacteria under anaerobic conditions in the presence of different concentrations of Fructooligosaccharides or Glucose, each separately. The number of bacterial colonies and the rate of sugar consumption were measured to determine the most efficient and effective bacteria for use in the manufacture of lactic fermentation fortified with Fructooligosaccharides. The results showed that the addition of Fructooligosaccharides had a clear effect in enhancing the growth of Lactobacillus paracasei, Lactobacillus plantarum, and Lactobacillus casei bacteria with growth rates of (5.204, 3.398, and 3.3160) cFu\ml compared to the growth rates in the medium treated with glucose only, which reached 3.564, (2.3980, and 1.8890) cFu\ml, respectively. On the other hand, depending on the extent of sugar consumption from the medium and the optimum incubation period with the highest sugar consumption, the growth efficiency of different types of bacteria differed according to the concentration of the growth medium of FOS, which was used at concentrations of (10, 7, 5, 3, and 1)%. The concentration of 5% recorded the highest growth rate of L. paracasei with the best incubation period of 32-48 hours, while the concentration of 1% was the most effective in increasing the growth of L. casei with an optimal incubation period of 16 hours, while the highest growth of Lactobacillus plantarum was achieved using FOS medium at a concentration of 10% and an incubation period of 32 hours.
**Keywords: Food bacteria, Fructooligosaccharide, fermentation,**

## Introduction

Fructooligosaccharide is one of the natural organic compounds found in fruits, vegetables, and whole grains. This sugar contains (2-10) monomeric units linked by a glycosidic bond of the type. (FOS) is widely used as a natural sugar with a sweetness of (30-50%) and is a low-calorie, indigestible and antibiotic-resistant compound [7]. This sugar passes through the small intestine and then to the large intestine, where these compounds stimulate the growth and activity of probiotics in the digestive system [16]. Lactobacillus plantarum bacteria play an important role in the digestion of sugars as a major carbon source in the coexistence and growth of bacteria within the culture medium. Probiotic bacteria are considered one of the first genera to have probiotic properties [3], as they are classified as non-pathogenic, non-toxic, acid-resistant bacteria that have many positive effects on human health. These bacteria have the ability to inhibit many types of pathogenic bacteria such as (Salmonella - Shigella), obligatorily homofermentative, and some of their strains are characterized by its ability to ferment starch, it has been isolated from human sources such as (intestine - mouth -

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reproductive system), dairy products, sourdough and alcoholic beverages [8].

The genus Lactobacillus is the main and most diverse group of lactic acid bacteria (LAB) with a focus on the species L. plantarum, due to its great adaptability. Lactobacillus acidophilus is one of the species that characterizes the genus Lactobacillus, and is characterized by its ability to withstand adverse conditions of the digestive system (low pH and bile salt toxicity) [11].

Several studies have reported that the intake of (FOS) can promote the growth of different types of Lactobacillus bacteria in the large intestine. Therefore, fructooligosaccharides are currently used as an alternative to antibiotics [5]. On the other hand, propionic acid (PA) has an inhibitory effect on the growth of mold and pathogenic bacteria. Propions such as sodium propionate and calcium propionate may also be used as food preservatives [6]. Therefore, the study was conducted with the aim of determining the optimum conditions and concentration of the nutrient medium for probiotic bacteria, especially by determining the best (pH), incubation duration, highest bacterial aggregation and lowest consumption of sugars.

Materials and methods

1- Preparation of the culture medium

The fermentation medium [1] was prepared using the following components: Peptone 2.5 g/L, dipotassium phosphate 0.5 g/L, Magnesium Sulfate hephahydrate 0.095 g/L, Magnesium Sulfate Monohydrate 0.0625 g/L, Tween 80, Anhydrous Sodium acitate 1.25 g/L, Diamononium hydrogen citrate 0.5 g/L, and Systeine 0.13 g/L. It was added to 100 ml of distilled water, distributed into 10 ml test tubes and sterilized automatically. After cooling to room temperature, the fermentation media were inoculated with bacteria and incubated at 37 °C for 24-48 h, after which the amount of sugar consumption by bacteria and the number of bacteria were measured.

For the purpose of calculating the logarithm of live starter bacteria, solid MRS Agar medium was used according to the manufacturer's instructions after sterilization with a bacteriological filter to the culture medium to ensure that the medium was free of oxygen [14]. Liquid MRS Broth medium was also prepared free of oxygen to activate the probiotics.

Determination of optimal laboratory conditions

The consumption of sugars by bacteria in the fermenter [9] was measured in 2 ml of the sample at a wavelength of 600 nm at room temperature, and the biomass was measured according to the method described by Zhang et al., (2024) [18]. also at a wavelength of 600 nm using a spectrophotometer.

The number of bacteria was also measured by a spectrophotometer at a wavelength of 600 nm in 2 microns of the sample and placed in a cuvette cell to determine the absorbance and monitor the number of bacterial cells and their growth development inside the medium after 48 h of incubation in an anaerobic carbon dioxide incubator to achieve optimal conditions.

Effect of adding concentrations of (FOS) to the culture medium on the growth and consumption of bacteria for sugar The method described by Shao et al. (2025) [15] was followed, as the chemical medium for fermentation was prepared by adding different concentrations of sugar at ratios of (1, 3, 5, 7, 10)% and inoculated with three types of bacteria (L. paracacia, L. casei, L. plantarum). The sample was withdrawn every 8 hours to test the growth of bacterial numbers and adjust

sugar consumption. The effect of changes in pH on bacterial numbers and sugar consumption was also tested by adjusting the pH of the culture medium to pH (5, 6, 7, 8) by adding HCL and NaOH. It was incubated for 48 hours under anaerobic conditions and samples were taken to measure bacterial numbers with their sugar consumption every 8 hours. The effect of incubation period (8-48 hours) on bacterial numbers and their sugar consumption was tested in the same way. The standard curve for fructooligosaccharide (FOS) and glucose was determined by measuring the absorbance at a wavelength of 490 nm. The first blank tube was used to zero the device, and then the standard curve was determined with the concentration of fructooligosaccharide and the absorbance reading, Figure (1).

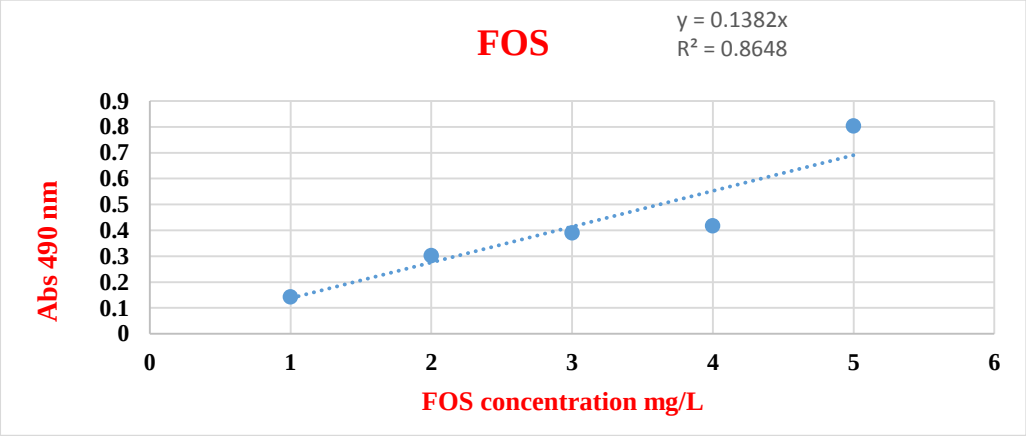


**Figure1. Standard curve of fructooligosaccharide (FOS) for estimating carbohydrate content**

Results and discussion

Effect of FOS concentrations on Lactobacillus growth

The results shown in Figure (2) indicate that the use of concentrations of FOS led to variation in the number of bacteria and the percentage of FOS consumption in the culture medium, as the concentration of 5% was more effective in the number of 1.249 cfu\ml of L. paracasei compared to the other FOS concentrations. On the other hand, 1% FOS led to the highest number of 1.219 cfu\ml of L. casei bacteria, while the number of bacteria was (0.580, 0.692, 0.175, 0.832) cfu\ml at FOS concentrations of 10, 7, 5, 3%, respectively, while, the highest number of bacterial colonies of L. plantarum was recorded using FOS at a concentration of 10%. FOS is an important carbon source for bacterial growth in addition to glucose. The presence of these carbon sources affects the growth of microorganisms by stimulating their growth or reducing the growth of microorganisms by using high or low concentrations. It affects the osmotic pressure inside the cells, which leads to the representation of high levels of sugars and thus increased growth [17]. On the other hand, sugars affect the course of action of a number of enzymes Glucose-6-phosphate dehydrogenase, Hexokinase, Glucose dehydrogenase. In addition to raising the work requirements for growth and fermentation and increasing the work of other elements. In general, FOS works on the molecular hydrolysis of fructose as a carbon source and energy source, which affects the process of

vital metabolism inside the cell, in addition to being a basic criterion for probiotic bacteria and its formation of fructose monomers [10].

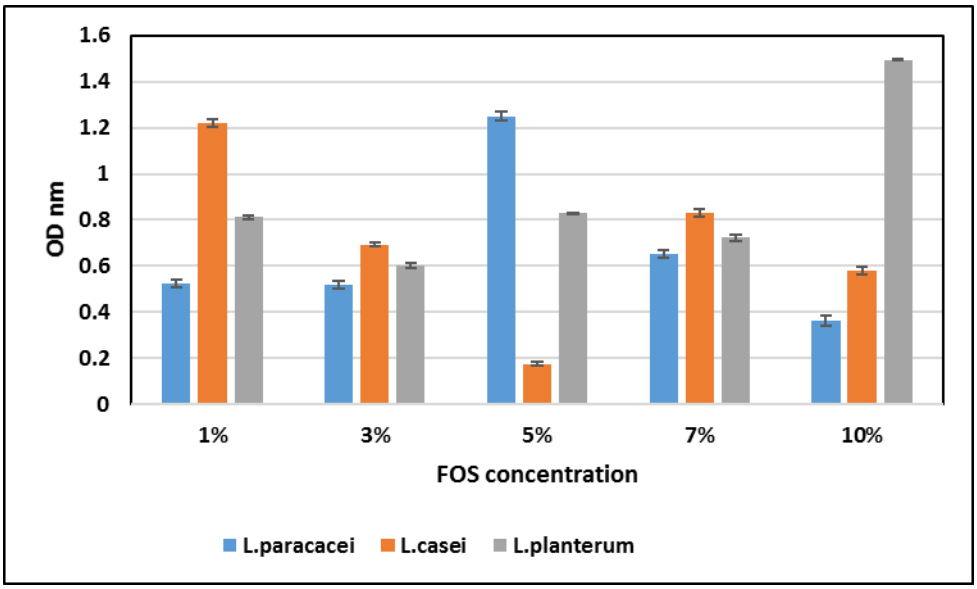


**Figure2. Effect of fructooligosaccharides FOS at different concentrations on growth and sugar consumption rate of three Lactobacillus species**

Effect of FOS concentrations on Lactobacillus bacteria sugar consumption rate

The results indicate that the highest consumption of FOS by L. paracasei was when using a concentration of 7% with a consumption rate of 2.700 g/L, which decreased at a concentration of 10% to 2.1750 g/L, while the other concentrations recorded lower sugar consumption rates, while the highest sugar consumption by L. casei was when using FOS at a concentration of 5%, respectively. As for using concentrations of FOS, sugar consumption was higher at a concentration of 3%, while sugar consumption by L. plantarum was more efficient at a concentration of 7%, which recorded 2.3500 g/L with a significant difference compared to the highest and lowest concentrations (Figure3).

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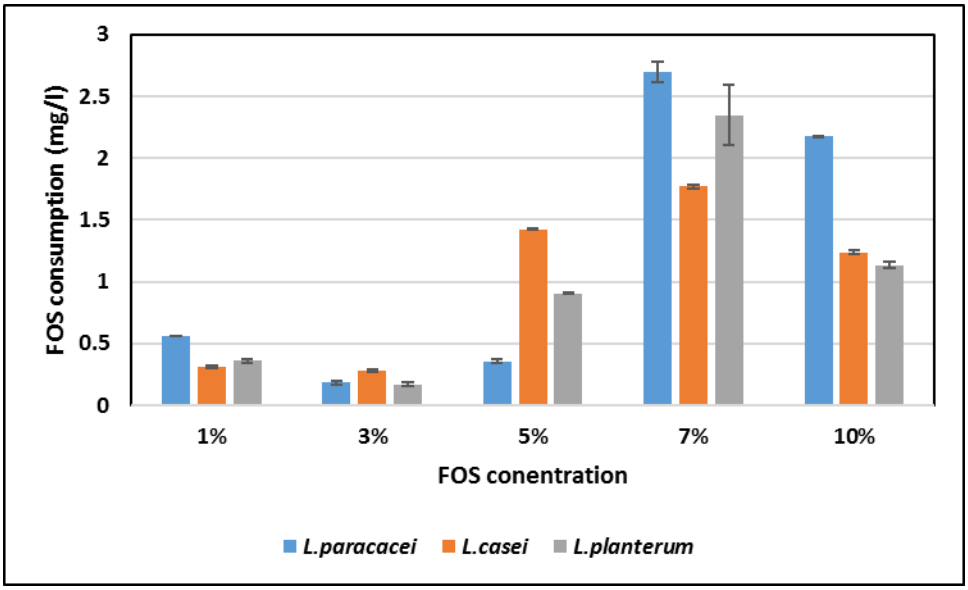


**Figure 3. Differences of three Lactobacillus bacteria in the ability of sugars consumption at different FOS concentrations**

Effect of incubation periods on Lactobacillus spp. growth and sugar consumption rate in culture medium treated with FOS concentrations

The results in Figure (4) indicate that the incubation period had an effect in increasing the bacterial numbers in the presence of different concentrations of FOS, L. paracasei bacteria recorded the highest bacterial count rate of 1.492 cfu\ml on FOS medium with an incubation period of (32) hours, while L. casei bacteria were more effective at an incubation period of (32, 48) hours, reaching (3.8487, 5.6767) cfu\ml. respectively. As for L. plantarum bacteria, it recorded the highest bacterial count rate of 1.482 cfu\ml after 32 hours, with a very slight difference from that recorded after 16 or 48 hours, and the lowest densities were recorded after 8 and 16 hours.

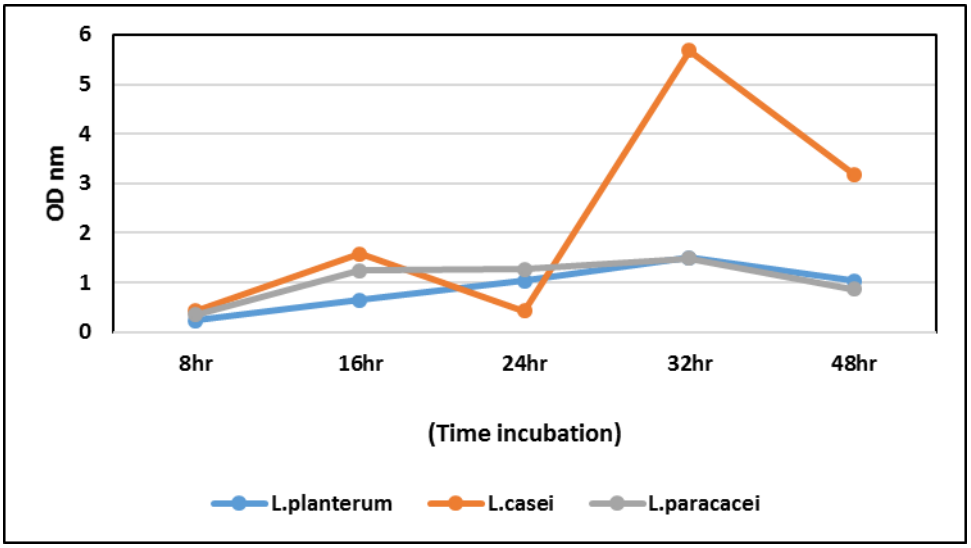


**Figure 4. Effect of incubation periods on Lactobacillus spp. growth in culture medium treated with FOS concentrations**

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The effect of the incubation period on the FOS sugar consumption rate was also observed, which differed according to the type of bacteria, as the highest sugar consumption rate was recorded at 16.705 for L. plantarum bacteria at an incubation period of 48 h, while the highest sugar consumption for L. casei was 3.787 g/l after (16) hours of incubation, while L. paracasei recorded the highest sugar consumption rate at 48 h of incubation at 4.890 g/l (Figure 5). The variation in bacterial growth and sugar consumption rate within the culture medium is due to the variation in the bacteria's use of nutrients within the medium and the bacteria reaching the consumption stage. In addition to the accumulations resulting from the various metabolic materials within the medium, adding sugars to the culture medium as a carbon source affects the increase in the number of bacteria and their association according to the fermentation period, and 24 h is the best fermentation period for most organisms [12]. It was shown [4] that using EXP as a sugar source and L. fermention F.6 Bacteria during 24 h of fermentation recorded a sugar consumption of 153.8 g/L, while when using EXP at a concentration of 10% and a 24 h incubation period in the presence of Lactococus lactis bacteria, the amount of sugar consumed was 0.212 g/L. It was also reported by Cao et al. (2019) [2] that using different concentrations of Oligosaccharides affected the growth of L. plantarum bacteria during the 24 h incubation period, as the bacteria are examined for 3 h to compare with other sugars, while changing the pH also affected the growth of bacteria significantly.

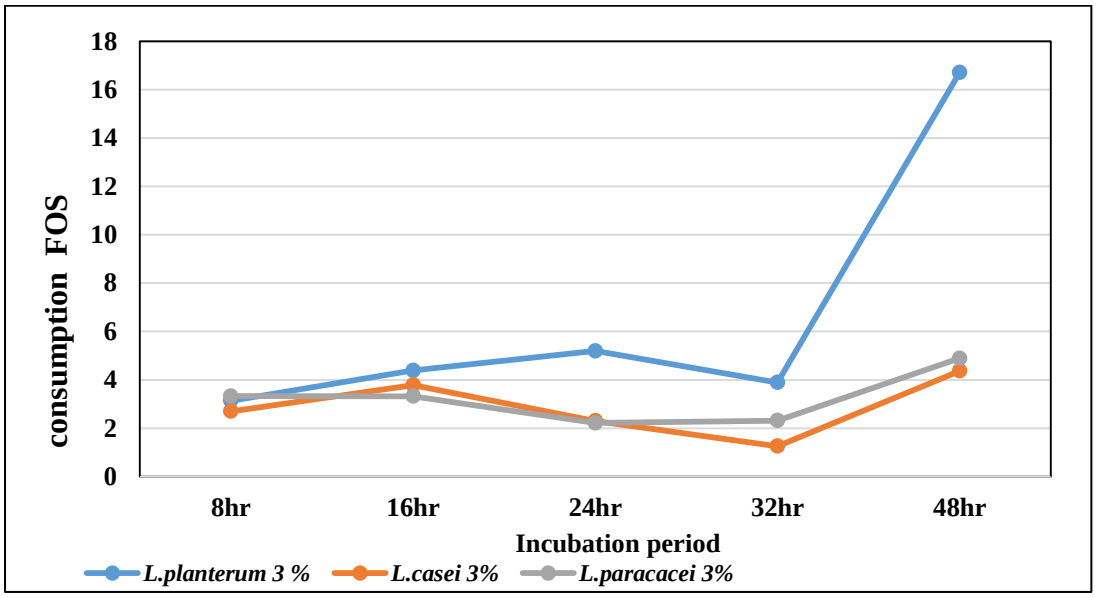


**Figure 5. Effect of FOS concentrations on Lactobacillus spp. sugar consumption rate**

Effect of pH on Lactobacillus spp. growth and sugar consumption rate in culture medium treated with FOS concentrations

The results shown in Figure (6) indicate that the pH value had a significant effect on the bacterial growth rate and sugar consumption rate. Generally, at (pH = 7) in the culture medium treated with FOS, the number of L. plantarum bacteria increased to 1.020 cfu\ml compared to (6.246, 0.346, 0.356) cfu\ml at

pH values = (5, 6, 7), respectively. As for L. casei bacteria, at pH = (6.3), the number of growing bacteria was 2.090, 0.2400 cfu\ml, while at pH = (7.8), the number of bacteria reached (0.400) cfu\ml. As for pH = (6.5), the sugar consumption rate was (4.573, 4.09) g\l, respectively (Figure 7).

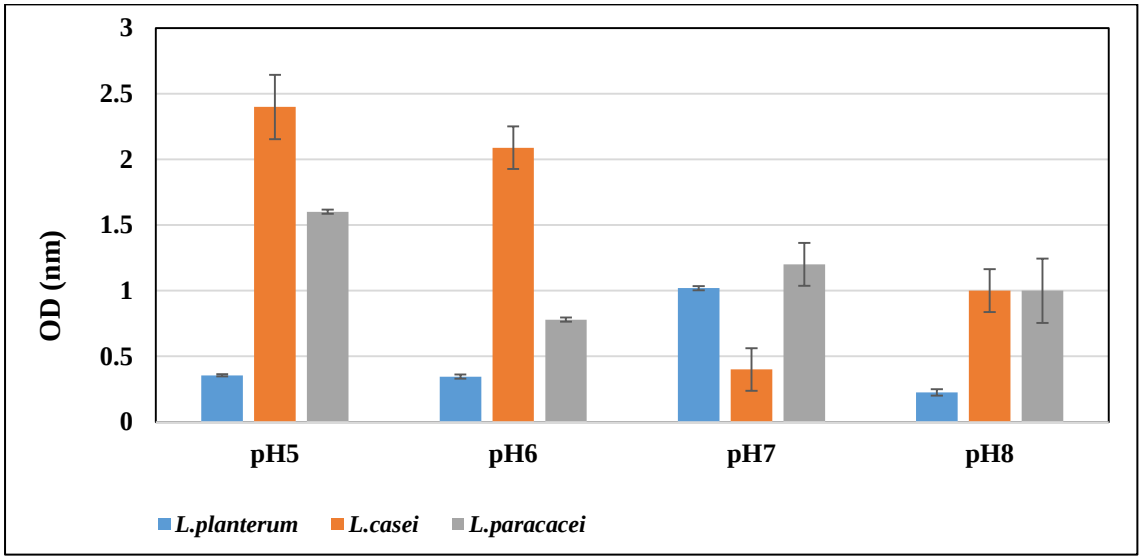


**Figure 6. Effect of pH on Lactobacillus spp. growth in culture medium treated with FOS concentrations**

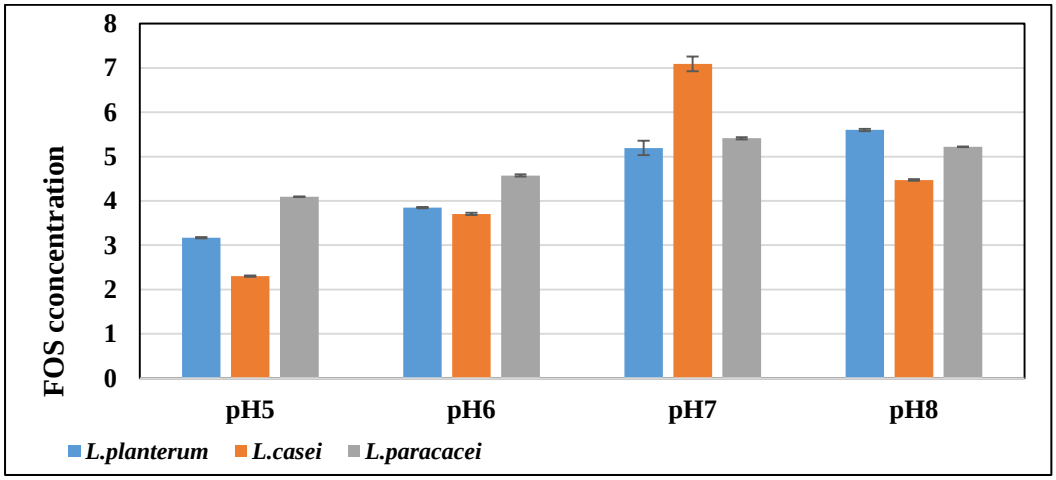


**Figure 7. Effect of pH on Lactobacillus spp. sugar consumption rate in culture medium treated with FOS concentrations**

The variation among Lactobacillus species in sugar consumption may be due to its effect on the enzymes responsible for metabolic processes inside the cells, in addition to its effect on the work of the cells and the production of proteins that contain ionized groups, the mechanisms of which are diverse inside the cells, which leads to a malfunction in the work of the cell, as the effect of its enzymes through its effect on the composition and mechanism of functional work inside the medium, which affects the percentage of sugar consumption and thus affects the work of the microscopic organism [13.[

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## Conclusion

Findings showed that bacterial growth medium prepared with Fructooligosaccharides at different concentrations enhanced the growth (number of bacteria colonies) of Lactobacillus paracasei, L. plantarum, and L. casei compared to lower growth rates in the medium treated with glucose only. On the other hand, bacteria growth and ability of sugar consumption differed among Lactobacillus species and was affected by the medium pH, and incubation periods. It can be concluded that Lactobacillus species especially L. plantarum can commercially propagated and effectively grown using culture medium prepared with Fructooligosaccharides rather than other sugar sources.

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