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Original Research Article

Detection and Environmental Optimization of dextran produced from *Lactobacillus* spp.

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Abstract: ***Introduction*:** Dextrans are D-glucose homopolysaccharides made by bacteria. There are several different bacterial species that produce these polysaccharides. Dextran is used as an adjuvant, drug carrier emulsifier, carrier, and stabiliser in the food, pharmaceutical, and chemical sectors, Nano gel. The aim of the study is to isolate *Lactobacillus* spp. from clinical sources, to identify the dextran production yield in *Lactobacillus* spp, and to reach the optimal growing parameters to discover the optimal dextran growth conditions. ***Methodology***: 50 isolates of *Lactobacillus* spp. were gathered from different medical facilities in Baghdad City, 21 isolates came from healthy women's vagina (16 *Lactobacillus plantarum*, 5 *Lactobacillus acidophilus*), and 29 isolates came from samples of infant stool (15 *Lactobacillus plantarum*, 4 *Lactobacillus acidophilus*, and 10 *Lactobacillus gasseri*). All isolates were examined for dextran production using mucoid and spectrophotometric methods. ***Results***: *Lactobacillus acidophilus* that was isolated from the genital tract of healthy women produced greater dextran. The results shows that the optimum conditions for dextran production that were investigated, there are several parameters play important roles to reach the optimal conditions, including, different natural carbon sources, nitrogen source, temperature, sucrose concentration, incubation time, pH, inoculum size. ***Conclusions***: he optimum conditions for production were at 37 ºC for 48h at pH 5.5 with 4 % inoculum size and 6 g/ 100ml sucrose concentration with 6% best nitrogen source beef extract and the best natural carbon source for dextran production was dates.

Keywords: Dextran, *Lactobacillus* spp., Baghdad.

INTRODUCTION

Lactobacillus is a taxonomically complicated genus that has around 170 species. The *Lactobacillus* genus includes bacteria that are rod-shaped, non-spore-forming, catalase-negative, microaerophilic to strictly anaerobic. The tier common culture characteristics includes temperatures between 30°C to 40°C are ideal for Lactic Acid Bacteria (LAB), while the pH range between 4.5 and 6.5 is ideal [1].

Lactobacillus breakdown carbohydrates and yield lactic acid as an end product therefore they are considered the largest genus that belongs to LAB group, *Lactobacillus* and humans and other animals have a mutualistic interaction. They live in numerous parts of the human body, most notably the GIT system and the female reproductive tract. Several *Lactobacillus* species give the host aid in digesting particular food substrates and defence against harmful microbes in exchange for residence and nutrients [2, 3].

Exopolysaccharide are long-chain, linear or branch biopolymers with a high molecular weight that are made up of often repeated saccharide units or saccharide derivatives connected by a- and b-glycoside bonds. EPS is secreted into the environment as slime (slime EPS) or adheres to the bacterial surface of the cell, forming a capsule (capsular EPS).

Exopolysaccharides are biological polymers that microorganisms including Lactic acid bacteria, secrete in order to endure harsh environmental conditions [3, 4].

Dextran is one of numerous EPS that has gained worldwide recognition for its biodegradability and biocompatibility [5]. Dextran is a bacterial homo-polysaccharide cationic polymer whose main chain is made up of several -glucans linked by -(1-6) glycosidic bonds with varying amounts of branched linkages such as -(1-2), -(1-3), and -(1-4) linked as a single unite or lengthened side chain, the degree of branching depending on the bacterial strain used for production. The helical shape of dextran's main chain with α-(1→6) bonds is modified by the presence of branches (α-(1→2), α-(1→3) or α-(1→4)), causing the linear structure of glucan to be folded repeatedly. Dextran is produced by the action of the enzyme dextransucrase in which microorganism start to synthesise when cultivated in an environment contains sucrose [6].

Dextran is a basic biological macromolecule whose origin is directly tied to both its molecular weight and degree of branching. It is simple to chemically alter dextran to create a variety of derivatives. Dextran has exceptional water solubility, biocompatibility, and biodegradability, Due to its basic and non-immunogenic bio-polymeric character, dextran derivatives, dextran conjugates, dextran hydrogel, and micelles are also widely employed as nanotechnology in medicine and Nano carriers. Dextran 70 which is a blood plasma replacement is listed on the WHO model list of essential medications as of April 2015 [7].

The aim of the study include isolation of *Lactobacillus* spp. from clinical sources, screening for dextran production by *Lactobacillus* spp, isolates acquired, and reach the optimal growing conditions for dextran synthesis by the chosen isolate.

METHODOLOGY

Collection of bacterial isolates

88 clinical bacterial samples were collected from various hospitals and clinics in Baghdad city, out of these 88 samples there were 53 samples from human vagina and 35 from new-borns stool, after collection, all the bacterial isolates were identified upon on their colony characteristics, microscopic inspection, biochemical tests and VITEK 2 compact system.

Dextran production

Mucoid method

The dextran production medium was prepared by adding by (g/l): Sucrose, 60; yeast extract, (10), $CaCl_2$ (0.14), $MgSO_4$(0.04) $FeSO_4$(0.04) $MnSO_4$ (0.02) NaCl (0.01), H_3PO_4 (5.7) and 15g agar powder to 100ml distilled water the PH was accustomed to 5.0 then the medium was autoclaved for the purpose of sterilization. Finally the medium was inoculated with 24 h old culture of *Lactobacillus* spp. Isolates, incubated for 24 h at 37 ^oC, the ropy mucoid appearance of isolates was documented as positive dextran producer [7].

Spectrophotometric method

The spectrophotometric technique was used to determine the dextran of selected *Lactobacillus* spp. isolates that were reported as producer isolates. Dextran production medium without agar - agar was inoculated with a *Lactobacillus* spp. solution. The medium was inoculated with 2% bacterial culture containing (9x 10^8cfu/ml) (matched to McFarland standard 0.5 ml absorbance at 600nm around 0.134), incubated at 37^oC for 24hrs. Following the incubation, the culture media was centrifuged for 10 min at 10,000 rpm, the biomass was removed, and the supernatant was used to calculate of dextran concentration [7, 8].

Determination the Optimal Conditions for Dextran Production

Effect of natural carbon sources

The selected bacterial isolate was inoculated to a dextran production medium containing several plant extracts at 6% as sources of carbon for dextran production, including date and beetroot, peach, pear, tangerine, apple, honeydew, watermelon, sweet potato, pineapple, and orange. Following the incubation period (48 hours at 37^oC), the dextran concentration for each carbon source was measured.

Effect of sucrose concentrations

The selected isolate of bacteria was inoculated into dextran production medium containing different quantities of sucrose (3, 4, 5, 6, 7, 8, and 9 mg/100 ml). Following a 48-hour incubation period at 37^oC, the dextran concentration, for each carbon source concentration was determined.

Effect of nitrogen sources

To find the most effective nitrogen source for dextran production, dextran production medium was supplemented with several types of inorganic and organic nitrogen sources (peptone, tryptone, beef extract, yeast extract, ammonium chloride, ammonium sulphate, and ammonium persulfate), then incubated at 37°C for 48 hours soon after being inoculated with the selected bacterial isolate. Dextran concentration was measured after incubation for each nitrogen source concentration.

Effect of nitrogen source concentration

The selected bacterial isolate was transferred to a dextran production medium that contained various concentrations of the best nitrogen source, such as (1–10 g/100 ml), and after 48 hours of incubation at 37°C, the dextran concentration, for each nitrogen source concentration were measured.

Effect of inoculum size

The selected isolate was introduced to the dextran production medium and cultured at different inoculum concentrations between (1 and 10%). Following incubation, the dextran concentration for each inoculum size was determined.

Effect of temperature

The chosen isolate was then inoculated to a dextran production medium. Following incubation time at various temperatures (4, 15, 25, 30, 37, and 40°C), the dextran concentration and dextran production yield for each temperature were determined.

Effect of incubation time

The selected isolate was added to the dextran production medium and incubated for different periods of time (24, 48, 72, and 96 hours). Dextran concentration, were assessed following each period of incubation.

RESULTS AND DISCUSSIONS

Collection of bacterial isolates

The results revealed that the majority of *Lactobacillus* spp. isolates were isolated from infant stool with (29) isolates and human vagina with (21) isolates, as shown in figure (1).

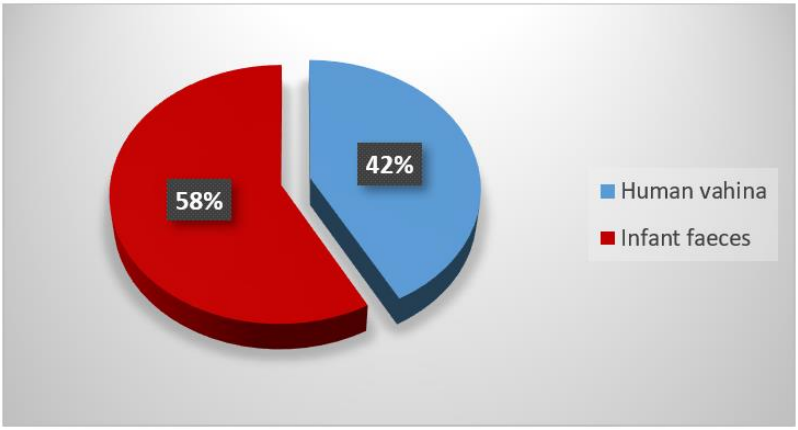

Figure 1: Collected *Lactobacillus* spp. isolates depending on the source of isolation

On the contrary hand, it is clear from the data in table (1) that the majority of *Lactobacillus* spp. 31 *Lactobacillus plantarum* isolates, 16 from healthy women's vagina and 15 from new-born faeces.9 isolates belonged to the *Lactobacillus acidophilus*, which consisted of 5 isolates from human vagina and 4 isolates from new-born faeces. In opposition, 10 *Lactobacillus gasseri* isolates were from infant stool.

Table 1: Distribution of *Lactobacillus* spp isolates according to source of isolations

Bacterial isolates	**Source of isolations**	
	Vagina of Healthy women	**Newborn infant Feces**
Lactobacillus plantarum	16	15
Lactobacillus acidophilus	5	4
Lactobacillus gasseri	-	10
Total	**21**	**29**

Screening of dextran producing isolates

The capability of *Lactobacillus* spp. isolates to produce dextran was investigated using mucoidy and spectrotrophotometric methods:

Mucoidy method

All 50 *Lactobacillus* spp. isolates were examined for dextran formation. Slimy mucoid colonies on the surface of dextran screening media were used to identify and screen dextran formation. Results showed in the table (2) that only 29 isolates were slimy mucoid colonies, 12 isolates from 29 isolates were produce strong slimy mucoid colonies, and 8 isolates from 29 isolates were produce moderate slimy mucoid colonies and 5 isolates were weak mucoid colonies.

Table 2: Screening for dextran Production by *Lactobacillus* spp.

Bacterial isolates	Viscosity
L plantarum V1	++
L plantarum V2	-
L plantarum V3	-
L plantarum V4	++
L plantarum V5	-
L plantarum V6	+
L plantarum V7	++
L plantarum V8	+
L plantarum V9	+
L plantarum V10	+++
L plantarum V11	++
L plantarum V12	+++
L plantarum V13	-
L plantarum V14	-
L plantarum V15	+
L plantarum V16	+
L acidophilus V17	+++
L acidophilus V18	+++
L acidophilus V19	+++
L acidophilus V20	+++
L acidophilus V21	+++
L plantarum S1	+
L plantarum S2	+
L plantarum S3	++
L plantarum S4	+
L plantarum S5	-
L plantarum S6	-
L plantarum S7	++
L plantarum S8	+
L plantarum S9	+++
L plantarum S10	++
L plantarum S11	-
L plantarum S12	-
L plantarum S13	-
L plantarum S14	++
L plantarum S15	-
L acidophilus S16	++
L acidophilus S17	+++
L acidophilus S18	+++
L acidophilus S19	++
L gasseri S20	-
L gasseri S21	+
L gasseri S22	+
L gasseri S23	++
L gasseri S24	-
L gasseri S25	+

Bacterial isolates	Viscosity
L gasseri S26	+
L gasseri S27	-
L gasseri S28	-
L gasseri S29	-
(+++): high production of dextran, (++): moderate production of dextran, (+): low production of dextran, (-): no production of dextran	

Polysaccharides are a key component in the building of the extracellular biofilm matrix. They are essential not only for shielding bacteria from harmful environmental conditions, but also for microbial cell adhesion to solid surfaces [9]. The screening techniques for evaluating microbial capacity to create EPS are based on growing the LAB on a medium supplemented with various sugars (glucose, fructose, sucrose, galactose, or lactose). Visually observing the phenotypic traits of the colonies is the simplest technique to determine EPS production: viscous or ropy appearances [10]. Slimy colonies are characterised by mucilaginous colonies, whereas ropy colonies are distinguished by the production of long filaments when an inoculation loop is pulled from the colony surface or cell pellet [11].

Spectrophotometric method

Following the selection of *Lactobacillus* spp. isolates that produced dextran (mucoid) at high levels; quantitative methods were used to assess dextran concentration. In this method 10 *Lactobacillus* ssp. isolates were picked out of 35 isolates that gave higher mucoid production. These 10 isolates showed different levels dextran production concentrations ranged between (0.56 - 0.9 mg/ml) with maximum dextran concentration 0.9 mg/ml by *Lactobacillus acidophilus* V19 as shown in figure (2).

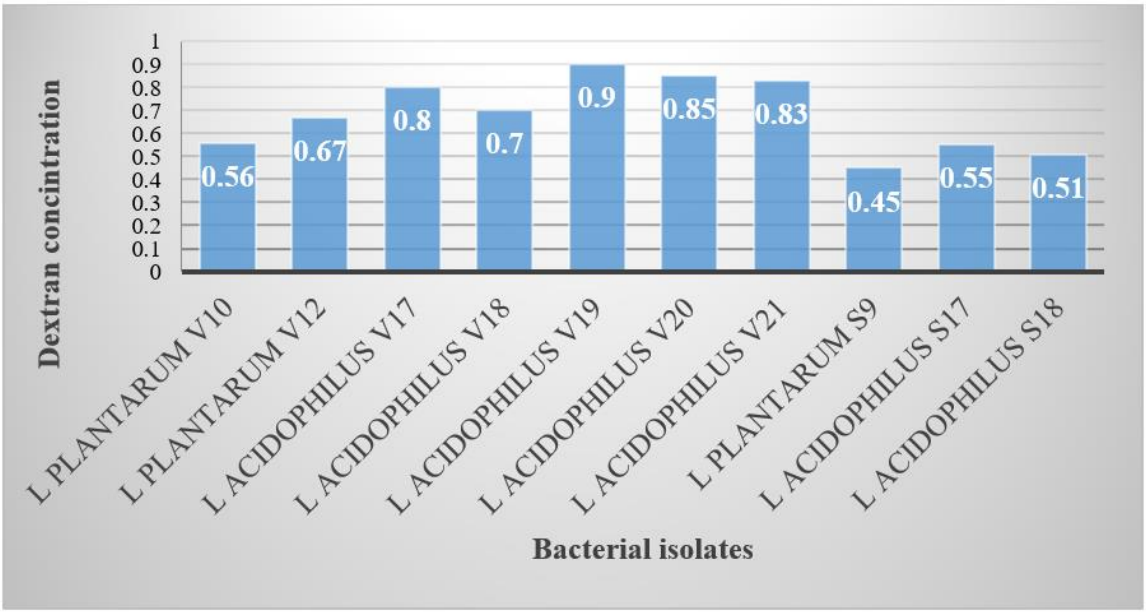

Figure 2: Concentrations of dextran produced by *Lactobacillus acidophilus*

Optimization of growth conditions for dextran production

Effect of natural carbon sources

Twelve different natural carbon sources were used in order to establish which one was the best for *L. acidophilus* V16 dextran production. Figure (3) from the data shows that among the twelve other naturally occurring sources of carbon, dates were the most effective carbon source, followed by sweet potatoes and oranges. The dextran concentration after date incubation was 1.2mg/ml. However, all other natural carbon sources reduced the amount of dextran produced, with carrots and honeydew producing the least amount at (0.51mg/ml) and (0.5mg/ml), respectively.

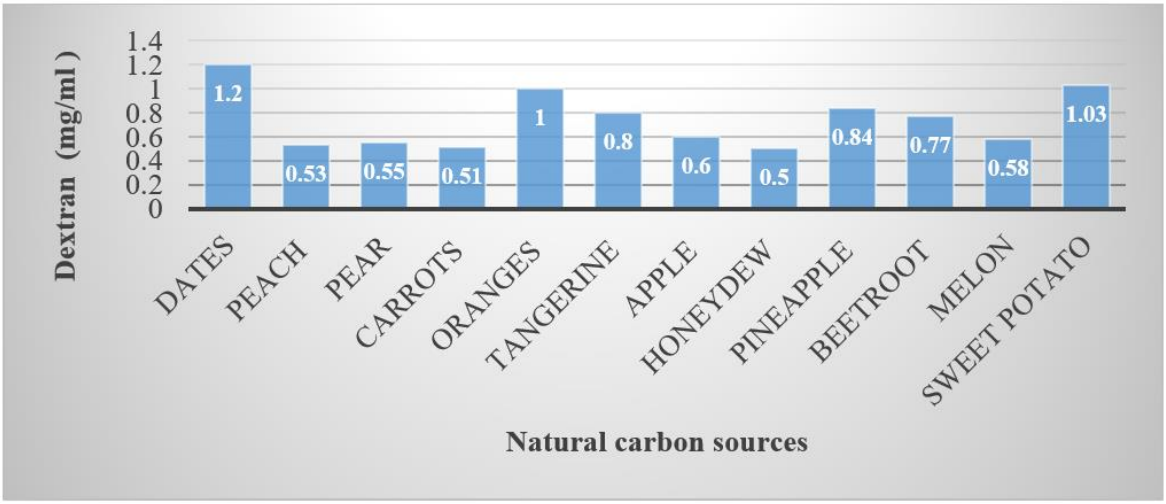

Figure 3 :Dextran production by *Lactobacillus acidophilus* V16 at different carbon sources

The amount of EPS produced by *L. acidophilus* V16 in the date's medium was high, according to EPS production. Based on the location of the fructose bond, different plants and microorganisms make fructans, which are fructose polymers. Dextran is one of the fructooligosaccharides produced in their investigations using date extract. Carbon is a resource that microorganisms use to develop and produce dextran. One of the crucial elements that affect the amount and quality of dextran synthesis is the carbon source. Dextran manufacture uses a variety of carbon sources, including sucrose, glucose, fructose, galactose, maltose, and mannitol [12].

Effect of sucrose concentrations

The optimal sucrose concentration for *Lactobacillus acidophilus* V16 dextran production was determined using a range of sucrose concentrations that included 1, 2, 3, 4, 5, 6, 7, 8, and 9%. The results as showed in figure (4) that 6% sucrose was the ideal amount of sucrose for the production of dextran, with 1.3mg/ml of dextran. In contrast, 9% sucrose reduced dextran concentration to 0.3mg/ml.

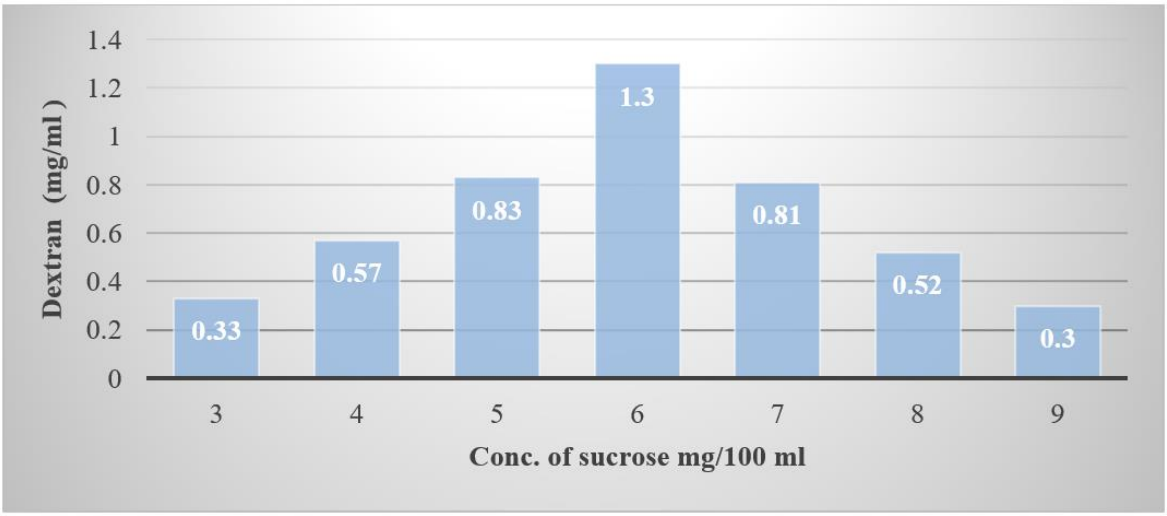

Figure 4: Dextran production by *Lactobacillus acidophilus* V16 at different concentrations of sucrose

The quantity of substrate has an effect on how much dextran is produced; researchers have previously shown that sucrose between 10% and 20% generates the most dextran because sucrose inhibits the production of EPS. The variations in molecular weight, types and numbers of branches in each dextran are relied on the producing strain (or enzyme) and the fermentation (or synthesis) environment, make each glucan complex and distinctive [13].

Effect of nitrogen sources

Using a dextran production medium, the impact of different nitrogen sources on dextran synthesis was investigated. The results appeared to be as shown in figure (5), which indicated that beef extract is the greatest organic nitrogen source for inducing dextran with concentrations reached to (1.5mg/ml), while, yeast extract was the next most

effective nitrogen sources for the production of dextran, while ammonium chloride, ammonium sulphate, and ammonium persulfate significantly decreased dextran production.

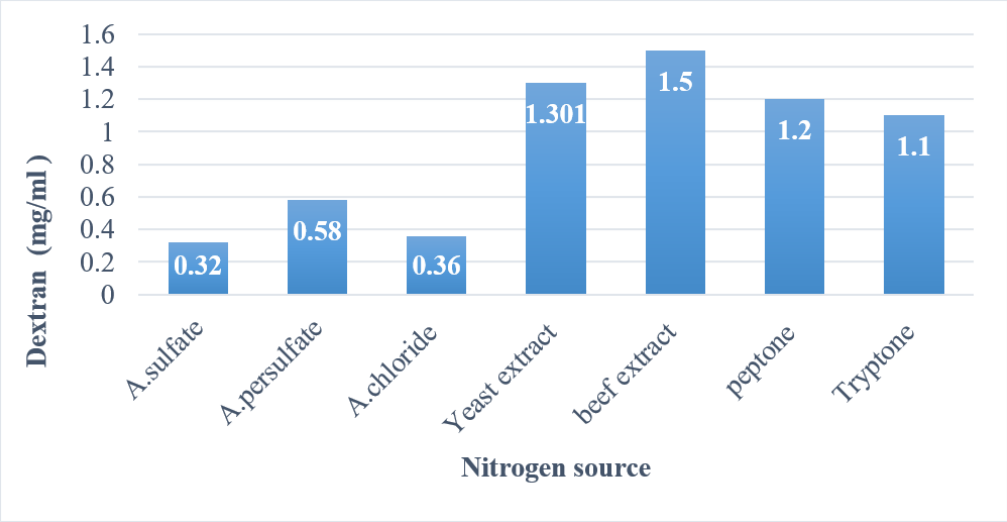

Figure 5: Dextran production by *Lactobacillus acidophilus* V16 at different nitrogen sources

The significance of nitrogen supplies for microbial cell development is second following the significance of carbon [14], nitrogen is an essential component of protein, nucleotides, enzymes, and a cofactor that is crucial to cellular metabolism. However, because dextran is an EPS, it must be produced by an enzyme called dextransucrase. Protein synthesis (enzyme dextransucrase) for dextran yield may be impacted by varied nitrogen sources' changing amino acid content [15].

Beef extract powder is dried meat extract that has been ground into a fine powder. An extract that is nutrient-rich and utilized to make a variety of culture media for the growth of various microorganisms. It provides a supply of nitrogen, vitamins, amino acids, and carbon for the microbiological growth medium, also, beef extract contains some amount of sugar that might also have contributed to the higher production of dextran [16].

Effect of nitrogen source concentrations

Using a range of concentrations of the beef extract ranging from 1 to 10%, the ideal concentration of the chosen nitrogen source for the synthesis of dextran was discovered. The results in figure (6), when production was at its peak, showed that the dextran concentration reached 1.65 mg/ml at a 6% concentration of beef extract. Increased beef extract concentrations significantly decreased the amount of dextran produced.

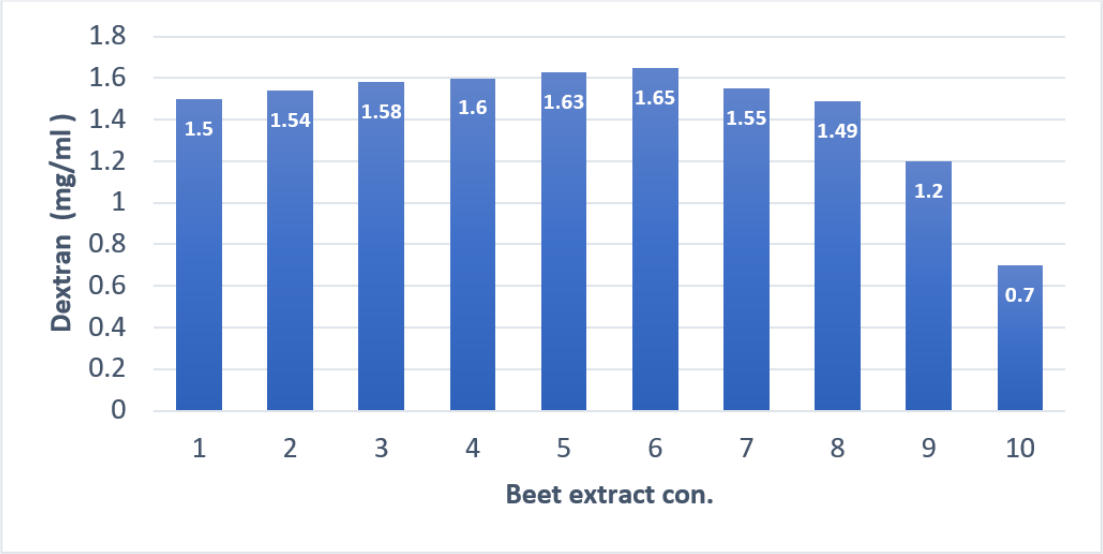

Figure 6: Dextran production by *Lactobacillus acidophilus* V16 at different concentrations of beef extract

Effect of inoculum size

The effect of inoculum size on dextran synthesis was studied. The *Lactobacillus acidophilus* V19 culture was incubated using a range of inoculum sizes (1–10%). The results showed that the optimal inoculum size for the production of dextran was at 4%, with dextran concentration (1.7mg/ml) and as inoculum size was increased, dextran concentrations dropped to 0.6 mg/ml as revealed in figure (7).

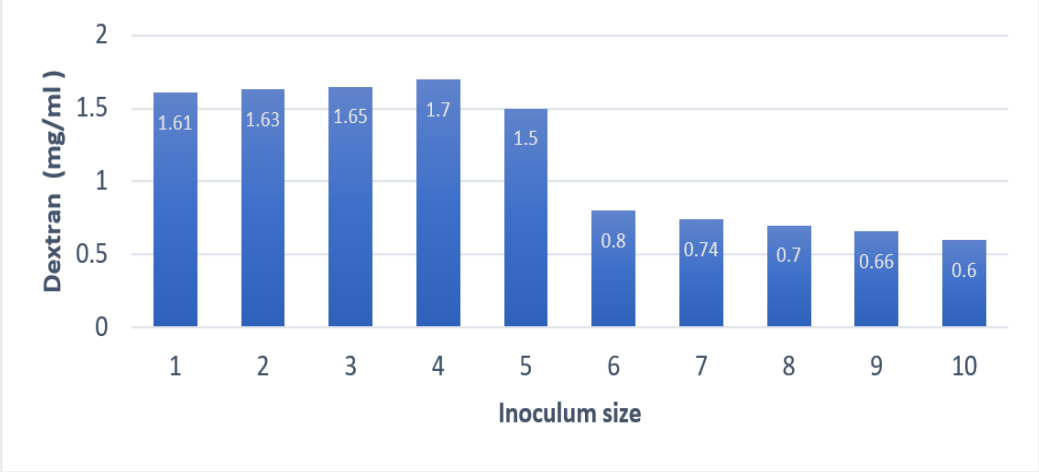

Figure 7: Effect of inoculum size on dextran production by *Lactobacillus acidophilus* V19

As the inoculum size grows, so will the interaction between the substrate, nutrients, and bacteria, which will enhance enzyme activity, protein synthesis, and bacterial metabolism. The enormous amount of inoculum size, on the other hand, results in competition for nutrients and substrates as well as a decrease in enzyme activity. As a result, competition from dense populations of quickly developing organisms and the early exhaustion of the medium's nutrients may be to blame for the reduction in activity with larger inoculum size. The high culture growth also causes oxygen loss [17].

Effect of PH

Dextran synthesis was uneven across pH ranges. Lactobacillus acidophilus V19 produced dextran at a pH of 5.5, with a dextran concentration of 31.46mg/. Figure (8) depicts further pH measurements that lowered dextran concentration and dextransucrase activity.

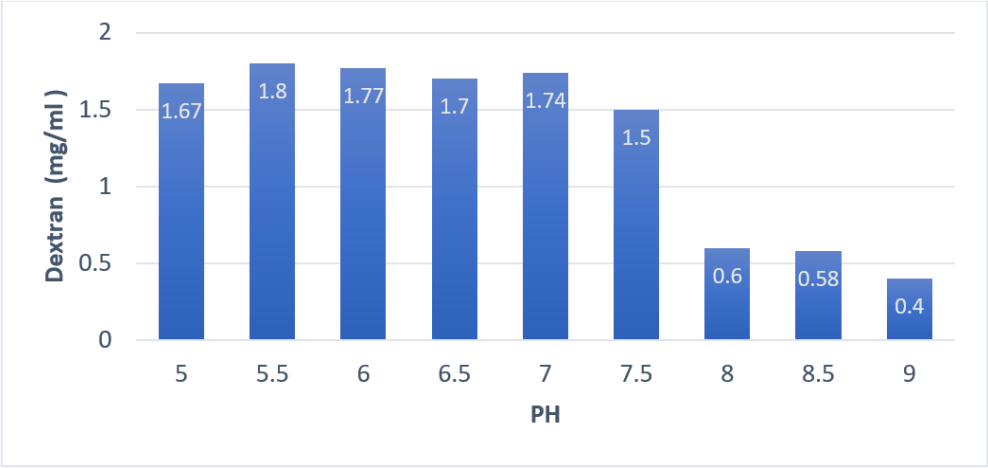

Figure 8: Effect of PH on dextran production by *Lactobacillus acidophilus* V19

Changes in the pH or acidity of the surroundings can affect or completely prevent the enzyme from catalysing a process. This pH change will affect the polar and non-polar intramolecular attractive and repulsive forces, as well as the enzyme configuration and the active site, to the point where the substrate molecule will no longer fit, and the chemical change will be inhibited or not occur at all [18].

Effect of incubation temperature

Several incubation temperatures (4, 15, 25, 30, 37, and 40°C) were investigated to discover the best one for *Lactobacillus acidophilus* V19 dextran synthesis. Highest dextran production occurred at 37°C.At this temperature, the dextran concentration was (1.8mg/ml). However, as seen in figure, greater and lower temperatures resulted in a reduction in dextran content as revealed in figure (9).

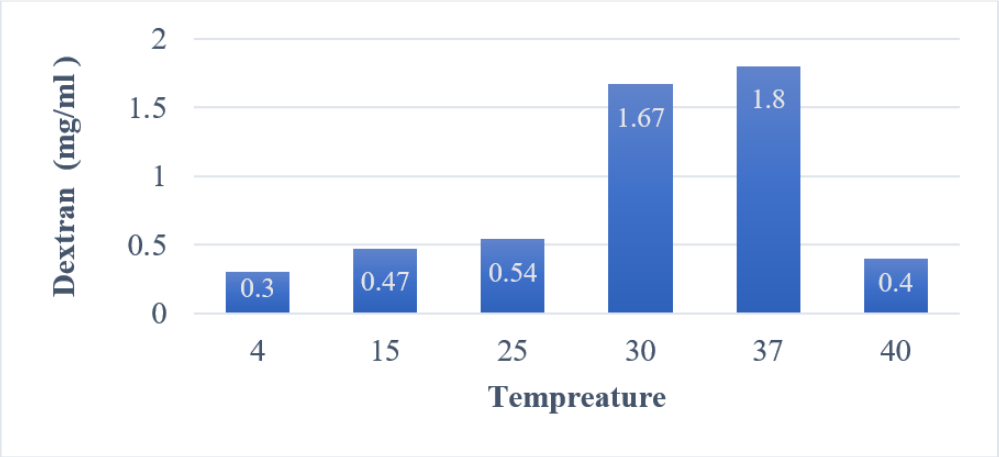

Figure 9: Effect of incubation temperature on dextran production by *Lactobacillus acidophilus* V19

Effect of different incubation time

Lactobacillus acidophilus V19 yielded (1.8mg/ml) after 48 hours of incubation, according to the results. The concentration dropped with increasing incubation time, reaching (0.4mg/ml) after 72 hours, however after 96 hours, the concentration began to diminish, as in figure (10).

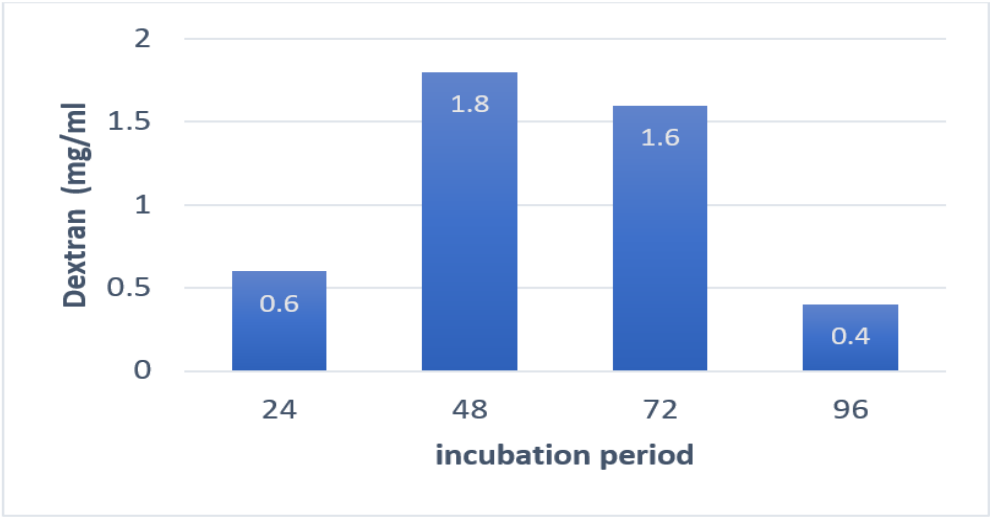

Figure 10: Dextran production *by Lactobacillus acidophilus* V19 at different incubation time

As the incubation time increase the nutrients depletes in the surrounding due to exponential growth of the bacterial population, plus, wastes and toxic by-products start to accumulates which would halt the biological processes within the cell? That might explain the decrease in enzymatic activity of the enzyme. Its also worth mentioning that those accumulated by-product can interfere between the enzyme and its substrate [19].

CONCLUSIONS

- *Lactobacillus acidophilus* isolates were stronger than *Lactobacillus plantarum* and *Lactobacillus gasseri* isolates in production of dextran.
- *Lactobacillus acidophilus* isolates that were isolated from human vagina were better than the *Lactobacillus acidophilus* isolates that were obtained from infant stool in dextran production,
- The optimum growth conditions for the best dextran production are: 2% dates extract as carbon source, 6% beef extract as nitrogen source, pH adjusted to 5.5 with 4% inoculum size. Incubation for 48 hours as 37°C

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