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Production and characterization of dextran from *Lactobacillus* sp. HH2 isolated from fermented cucumber

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Abstract: Dextran is a high molecular weight exopolysaccharide with diverse biomedical applications. Microbial synthesis of dextran using lactic acid bacteria (LAB) provides a scalable and safe alternative to conventional methods. In this study, seven LAB strains were isolated from fermented cucumber and screened for dextran production based on mucoid colony morphology and dextransucrase activity, as determined by the dinitrosalicylic acid (DNS) assay. From amongst the seven LAB isolates of fermented cucumber, dextransucrase activity ranged from 0.0139 to 0.0377 U/mL, with the highest activity from isolate L5 (Lactobacillus sp. HH2). This strain, identified as Lactobacillus species, was confirmed to be the producer of both the enzyme and the polysaccharide. Morphological, physiological, and biochemical analyses indicated acidogenic characteristics typical of the Lactobacillus genus, including catalase-negative and oxidase-negative properties. Dextran was precipitated using 98% chilled ethanol, further purified through sequential ethanol washes, and freeze-dried. Fourier-transform infrared (FT-IR) spectroscopy revealed characteristic α-(1→6) glycosidic linkages, while nuclear magnetic resonance (NMR) spectroscopy confirmed the polymer's structure with a dominant peak at 3.30 ppm (α-1→6), and secondary signals at 5.3 ppm and 4.03–4.11 ppm corresponding to α-(1→3) and α-(1→2,6) branches. These findings demonstrate the successful microbial production and structural characterization of a bioactive dextran polymer by a Lactobacillus sp. HH2 from fermented cucumber.
Keywords: Dextran, dextransucrase, Lactic Acid Bacteria, *Lactobacillus* ssp, FT-IR, NMR.

1. Introduction

Lactic acid bacteria (LAB) represent a phylogenetically diverse group of non-spore forming, Gram-positive cocci or rods that are typically facultative anaerobes or microaerophiles. They utilize fermentative pathways to convert carbohydrates into lactic acid via homofermentative or heterofermentative metabolism **[1]**. For instance, homofermentative species such as *Lactococcus lactis* convert glucose almost exclusively to lactic acid, while heterofermentative species like *Leuconostoc mesenteroides* produce lactic acid along with ethanol and CO_2 via the phosphoketolase pathway **[2, 3].**

Beyond their roles in food fermentation and preservation, LAB are increasingly recognized for their ability to produce functional biomolecules, including essential vitamins (e.g., folate, riboflavin, and B12 in co-culture systems), antimicrobial peptides (e.g., nisin, pediocin and plantaricin), and immunomodulatory metabolites **[4, 5]**. These capabilities enhance gut health, inhibit foodborne pathogens, and modulate immune responses **[6, 7]**.

The genus *Lactobacillus* is one of the most taxonomically and functionally diverse groups within the Firmicutes phylum **[6]**. This catalase negative, rod-shaped LAB utilizes fermentative metabolism to convert sugars into lactic acid, contributing to environmental acidification and competitive microbial exclusion **[7]**. Its metabolic adaptability and acid tolerance enable colonization of mucosal surfaces and persistence in acidic environments, making it integral to fermented food production and microbial therapeutics **[8]**.

Lactobacillus species exhibit ecological plasticity, being isolated from a wide range of sources including dairy products, fermented vegetables, sourdough, pickled fruits, gastrointestinal and vaginal tracts, and environmental niches such as soil and decaying plant matter **[9]**. Their genomic adaptations such as stress-response genes, carbohydrate transport systems, and acid resistance mechanisms support survival in variable pH and osmotic conditions **[8]**. These traits underlie their probiotic potential, immunomodulatory effects, and roles in host nutrient metabolism **[10]**.

Lactobacillus species are natural colonizers of various ecological niches, including the mucosal surfaces of the human and animal gastrointestinal (GI) tract, fermented dairy products, plant materials, and soil **[11]**. Their ubiquity in fermented foods such as yogurt, cheese, kefir, sauerkraut, kimchi, and sourdough bread is largely due to their acidification ability, which extends shelf life and enhances

food safety by inhibiting spoilage and pathogenic microorganisms **[2]**. The safety profile of LAB has been well-established, and many strains have received the "Generally Recognized as Safe" (GRAS) status from the U.S. Food and Drug Administration (FDA) or Qualified Presumption of Safety (QPS) status from the European Food Safety Authority (EFSA), which facilitates their use in food and pharmaceutical applications **[3]**. In addition to their classical roles in food preservation and flavor development, LAB have gained attention for their ability to produce a diverse array of functional and therapeutic biomolecules. These include essential vitamins such as folate, riboflavin, and B12 (especially in co-culture systems), which contribute to host nutrition and gut health **[4]**. LAB also synthesize antimicrobial peptides known as bacteriocins, such as nisin, pediocin, and plantaricin, which show strong activity against foodborne pathogens and are being explored as natural biopreservatives and therapeutic agents **[6]**. Furthermore, many LAB strains exert immunomodulatory effects by interacting with intestinal epithelial cells and dendritic cells, thereby influencing both innate and adaptive immune responses **[7]**. Perhaps one of the most promising avenues of LAB research in recent years involves their production of exopolysaccharides (EPS). These high molecular weight sugar polymers play a key role in microbial adhesion, colonization, and protection against environmental stressors; including pH shifts, osmotic pressure, and antimicrobial agents. The synthesis of EPS is genetically encoded and tightly regulated, and recent genome sequencing efforts have identified numerous EPS gene clusters across various LAB species, pointing to a wide range of structural diversity and functionality **[12]**. EPS from LAB have been shown to enhance the rheological properties of fermented foods, contribute to texture and mouthfeel, and function as dietary fibers with prebiotic effects that selectively promote the growth of beneficial gut microbiota, such as Bifidobacterium and Akkermansia **[13]**.

Moreover, LAB are emerging as platforms for synthetic biology and metabolic engineering aimed at producing high value bioproducts. Recent advances have enabled the use of CRISPR Cas systems and other gene-editing tools in LAB to enhance EPS production, improve stress tolerance, and optimize the biosynthesis of target compounds **[2]**. This metabolic plasticity, combined with their safety and fermentability, position LAB as next generation cell factories for the sustainable production of bio-based polymers, functional foods, and therapeutic molecules in both food and biomedical industries **[14]**.

Exopolysaccharides (EPS) are highmolecular weight carbohydrate polymers synthesized and secreted by many microbial species, including lactic acid bacteria (LAB), into their extracellular environment. Biopolymers, particularly microbial exopolysaccharides (EPS), are broadly classified into two types: homopolysaccharides (HoPS) and heteropolysaccharides (HePS) **[15]**. HoPS are composed of a single type of monosaccharide such as glucose or fructose and include examples like dextran and levan, typically synthesized by extracellular enzymes like glycosyltransferases **[13]**. In contrast, HePS consist of repeating units made from different monosaccharides, such as glucose, galactose, and rhamnose; and are produced through complex intracellular pathways involving sequential steps of sugar nucleotide synthesis, polymerization, and export. While HoPS often form simpler structures, HePS offer greater functional diversity, contributing to bacterial adhesion, biofilm formation, and environmental resilience **[16]**. The biosynthesis of EPS in LAB is a genetically-regulated process involving a coordinated cascade of glycosyltransferases, flippases, and polymerases; often encoded within specific eps gene clusters that vary by species and strain **[17]**.

From a functional perspective, EPS play a multifaceted role in microbial physiology and survival. In natural and host-associated environments, these polymers contribute to biofilm formation, cellular aggregation, and surface adhesion, offering protection against desiccation, oxidative stress, osmotic fluctuations, and antimicrobial agents. These properties are particularly advantageous in hostile niches such as the human gastrointestinal tract, where EPS-producing LAB exhibit enhanced persistence and probiotic efficacy **[18]**. Moreover, EPS contribute to the regulation of host immune responses. Some studies have demonstrated that HePS, due to their structural complexity, can interact with pattern recognition receptors (PRRs) on immune cells, leading to the activation or suppression of immune pathways depending on the EPS composition and conformation **[19]**.

In food biotechnology, EPS synthesized by LAB have garnered attention for their techno-functional benefits. These include improving the viscosity, texture, and water holding capacity of fermented dairy products such as yogurt, kefir, and cheese, as well as plant-based alternatives **[20]**. The use of EPS producing starter cultures in food fermentation has been shown to enhance the mouthfeel and structural integrity of products without the need for synthetic additives or stabilizers, aligning with current trends in clean-label food processing **[21]**.

Notably, the rheological properties of EPS are influenced by their molecular weight, branching degree, and charge density parameters that can be selectively modulated through strain selection or genetic engineering **[19]**. Recent investigations have also explored the health-promoting attributes of LAB-derived EPS beyond food texture enhancement. Many EPS exhibit prebiotic activity, selectively promoting the growth of commensal gut microbes such as Bifidobacterium longum, Faecalibacterium prausnitzii, and Akkermansia muciniphila; which are associated with improved gut barrier function and reduced systemic inflammation **[22]**. Additionally, in vitro and in vivo studies suggest that certain EPS from lactic acid bacteria have antioxidant, anti-inflammatory, antitumor, and

cholesterol-lowering effects, potentially mediated through direct interaction with intestinal epithelial cells, modulation of gut microbiota composition, and systemic immune signaling **[23]**.

Given their natural origin, biocompatibility, and structural diversity, EPS from LAB are increasingly being investigated for biomedical applications. These include their use in wound healing materials, vaccine adjuvants, and as scaffolds for tissue engineering. Furthermore, the chemical modifiability of EPS such as sulfation, carboxymethylation, or conjugation with bioactive agents has opened new avenues for their utilization as drug delivery carriers, especially for oral or mucosal routes where protective mucoadhesion is advantageous **[24]**.

Dextran is a water soluble, high molecular weight homopolysaccharide composed predominantly of α-(1→6)-linked D-glucopyranosyl units with varying degrees of branching through α-(1→2), α-(1→3), or α-(1→4) linkages. Originally discovered in *Leuconostoc mesenteroides*, dextran is synthesized extracellularly by the enzyme dextransucrase (EC 2.4.1.5), which catalyzes the polymerization of glucose from sucrose substrates. While *L. mesenteroides* remains the most studied producer, recent studies have confirmed dextran biosynthesis in other genera such as *Weissella*, *Lactobacillus*, and *Streptococcus*, broadening the biotechnological potential of dextran producing LAB **[25]**. These bacteria are valuable due to their Generally Recognized as Safe (GRAS) status, cost-effective cultivation, and ability to produce high-purity dextran without toxic by-products.

The therapeutic utility of dextran has also been extended to wound healing, tissue engineering, and immunomodulation. In surgical and trauma settings, dextran has been used to reduce postoperative adhesions and to act as a carrier matrix for growth factors and anti-inflammatory agents **[26]**. In addition, dextran-coated nanoparticles are under investigation as contrast agents in magnetic resonance imaging (MRI) **[27],** and as platforms for vaccine delivery, particularly for mucosal immunization **[28]**. With increasing interest in sustainable, microbially derived polymers, microbial dextran offers a compelling alternative to synthetic materials, aligning with the global push for bio-based therapeutics and biodegradable medical products **[29]**.

In this study, a dextran-producing *Lactobacillus* sp HH2 was isolated from fermented cucumber and is characterized by its biochemical properties and structural features. The goal was to assess the potential of this strain for dextran biosynthesis using a cost-effective natural source, and to evaluate the resulting polymer through advanced structural analyzes such as Fourier-transform infrared (FT-IR) and nuclear magnetic resonance (NMR) spectroscopy.

2. Materials and methods

2.1 Sample Collection

A total of seven vegetable samples, specifically cucumbers, were collected from various local markets in Sohag Governorate, Egypt. The purpose of this sampling was to enhance the likelihood of isolating lactic acid bacteria (LAB). Cucumbers were selected due to their potential to harbor plant-associated LAB, which are commonly present on raw vegetables and are known to contribute significantly to spontaneous fermentation processes **[30]**.

2.2 Sample Preparation

A fresh cucumber was obtained from a local market to investigate plant-associated lactic acid bacteria (LAB) involved in spontaneous fermentation **[21]**. The sample was placed in a sterile sampling bag, transported under controlled temperature conditions, and processed within 24 hours. A 10 g portion was aseptically transferred to a sterile glass jar containing 100 mL of sterile distilled water. The jar was loosely sealed and incubated at 37 °C for 24 hours to enrich LAB while limiting the growth of competing micro-organisms. The resulting culture was used for LAB isolation, with a focus on exopolysaccharide (dextran) producers **[31]**.

2.3. Isolation of Lactic Acid Bacteria (LAB)

LAB was isolated using **de Man, Rogosa, and Sharpe** (MRS) medium, which selectively supports the growth of lactic acid bacteria **[32, 33]**. Ten grams of the fermented cucumber culture were transferred into a sterile Erlenmeyer flask containing 40 mL of MRS broth and incubated at 37 °C under constant agitation (150 rpm) for 24 hours. To enhance LAB enrichment and reduce contamination by non target organisms, two successive transfers were made into fresh MRS broth under identical conditions. Following enrichment, 1 mL of the final culture was streaked onto MRS agar plates and incubated at 37 °C for 24 hours. Colonies exhibiting characteristic LAB morphology were selected for further purification and phenotypic characterization **[34].**

2.4. Identification of lactic acid bacterial isolates

Carbohydrate fermentation profiles were determined using phenol red broth containing individual sugars (glucose, lactose, sucrose, and maltose), enabling differentiation between homofermentative and heterofermentative species based on acid and gas production patterns **[35]**. Catalase and oxidase activities were evaluated using standard assays, providing additional taxonomic markers consistent with LAB characterization **[36] [37]**. Colony morphology and growth characteristics were observed on MRS agar following incubation at 37 °C for 24–48 hours. Cellular motility was examined via the hanging drop technique under phase-contrast microscopy, as described by Schillinger and Lücke **[38]**. Final identification of the isolates was based on

integrated morphological, physiological, and biochemical traits, following the classification criteria outlined in **Bergey's Manual of Systematic Bacteriology [39]**.

2.5 Viscosity Assay to Dextran Production

In the present study, lactic acid bacteria (LAB) were evaluated for their ability to produce dextran by assessing the viscosity of culture broths enriched with sucrose. Selected LAB strains were aseptically inoculated into sterile MRS broth supplemented with 10% (w/v) sucrose, in volumes ranging from 5 to 10 mL, to induce dextran biosynthesis via activation of dextransucrase enzymes, which are typically upregulated in the presence of sucrose **[40]**. The cultures were incubated at temperatures between 30°C and 37°C for 24 to 48 hours, depending on the optimal growth conditions of the specific strains, under either aerobic or anaerobic conditions to reflect their physiological requirements. Following incubation, each broth culture was visually inspected for changes in physical consistency.

2.6 Determination of dextransucrase activity

Dextransucrase activity was measured by the release of reducing sugars as dextran is produced, as described by the Miller test **[41]**. To prepare dextransucrase (DS), the whole bacterial strain was cultured in advance in 10 mL MRS broth supplemented with 2% (w/v) sucrose at 37°C for 24 hours. Following this, the culture was transferred to 40 mL fresh MRS broth supplemented with 2% sucrose and cultured under similar conditions for another span of 24 hours. After culturing, the centrifugation was performed on the culture broth under 6,000 rpm for 10 minutes at 4°C to procure separate cells, and the cell-free supernatant thus obtained was collected for measurement of the enzyme activity. A UV Vis spectrophotometer estimated the level of production of reducing sugars by measuring absorbance at 540 nm.

Dextransucrase activity was assessed by DNS method. A glucose standard calibration graph in the range of 0.2–1.0 µmol was constructed, with the linear relation $OD_{540} = 3.00 \times$ [Glucose, µmol]. A unit (U) dextransucrase activity was that enzyme level that under conditions specified in this assay released 1 µmol glucose equiv per min. Activity was calculated as U/mL crude extract, considering the volume in all experiments that was used as 1 mL enzyme.

2.7 Optimization of Dextransucrase Activity

The LAB isolate that showed the highest dextransucrase activity in the initial screening was selected for optimization studies. A series of experiments were conducted to determine the optimal physical and chemical conditions for maximum dextran production, including temperature, incubation duration, initial pH, sucrose concentration, and aeration. Enzyme activity was determined as described in the previous (section **2.6)**.

2.7.1 Effect of Temperature

To evaluate the effect of temperature on dextransucrase activity, cultures were incubated at 27, 30, 37, 40, and 45°C for 24 hours. Enzyme activity was evaluated after incubation to determine the optimal temperature.

2.7.2 Effect of Incubation Duration

The effect of incubation duration was evaluated by incubating cultures for 24, 48, and 72 hours under constant conditions. Enzyme activity was determined.

2.7.3 Effect of Initial pH

The initial pH of the production medium was adjusted to 3.0, 4.0, 5.0, 6.0, 7.0, 9.0, and 11.0 prior to inoculation. Cultures were incubated at the pre-determined optimum temperature and time to evaluate the effect of pH on dextransucrase activity.

2.7.4 Effect of Sucrose Concentration

To determine the optimum sucrose level for dextransucrase activity, sucrose concentrations of 2%, 4%, 6%, 8%, 10%, 12%, or 14% (w/v) were added to the medium. All other parameters were maintained at their pre-determined optimum conditions.

2.7.5 Effect of Aeration

The effect of oxygen availability was tested by incubating cultures under aerobic and anaerobic conditions. Aerobic cultures were stirred at 50 rpm to promote oxygen diffusion, while anaerobic cultures were incubated in closed, oxygen-free environments by using rubber plugs. All other conditions were kept constant.

2.8 Production of Dextran

Following optimization of dextransucrase activity, the most efficient cucumber-derived LAB isolate was selected to assess dextran biosynthesis. The isolate was first cultured in 10 mL of sterile MRS broth and incubated statically at 30 °C for 24 hours to promote active growth and metabolic activation **[42]**. This initial culture served as the primary inoculum.

The entire 10 mL culture was aseptically transferred into 100 mL of fresh sterile MRS broth in a conical flask and incubated under identical conditions. The culture was then successively scaled up to 500 mL and subsequently to

1000 mL. This stepwise expansion was employed to generate sufficient biomass for dextran production, as inoculum scaling has been reported to enhance both bacterial viability and enzyme activity **[18].**

2.9 Precipitation and Purification of Dextran

Dextran was recovered from the fermentation broth using ethanol precipitation, a widely used method for polysaccharide isolation due to its efficiency in reducing solubility and promoting selective aggregation **[37]**. Ice-cold 98% ethanol was added directly to the culture in a 3:1 (ethanol: culture) ratio under continuous stirring to ensure homogeneous mixing and effective polysaccharide precipitation. The mixture was then centrifuged at 6,000 rpm for 15 minutes at 4°C to separate the precipitated dextran from the supernatant.

To enhance purity, the precipitation process was repeated three times, as multiple ethanol precipitation steps have been reported to effectively eliminate non-dextran impurities, including residual proteins, oligosaccharides, and other extracellular metabolites **[43]**.

Following ethanol precipitation, the dextran pellet was subjected to a purification process to remove residual impurities such as cell debris, proteins, and media components. The precipitated dextran was first collected by centrifugation at 6,000 rpm for 15 minutes at 4°C, and the supernatant was discarded.

To further purify the dextran, the pellet was washed multiple times with cold distilled water to eliminate remaining contaminants. Each washing step involved resuspending the precipitated dextran in distilled water under gentle stirring to ensure uniform dispersion, followed by additional centrifugation to separate purified dextran from residual impurities **[44, 45]**.

Following ethanol precipitation, the purified dextran was subjected to freeze-drying (lyophilization) to ensure complete moisture removal while maintaining its structural and functional properties, thus preserving the polymer's molecular weight and physicochemical characteristics **[45]**.

2.10 Structural Characterization of Purified Dextran

The structural features of dextran produced by isolate from cucamber1 were characterized using Nuclear Magnetic Resonance (NMR) and Fourier Transform Infrared (FT-IR) spectroscopy, following established protocols **[45]**.

2.10.1 Nuclear Magnetic Resonance (NMR) Spectroscopy Analysis

For NMR analysis, both ^{1}H and ^{13}C spectra were obtained using high-resolution NMR spectroscopy. Dextran sample (30 mg) was dissolved in deuterium oxide (D_2O), lyophilized, and redissolved in fresh D_2O prior to analysis. The resulting spectra provided detailed insights into glycosidic linkages and the carbon backbone of the polysaccharide structure **[46]**.

2.10.2 Fourier Transform Infrared (FT-IR) Spectroscopy Analysis

Complementary FT-IR analysis was performed to identify functional groups associated with the dextran molecules. Sample was dried, ground, and analyzed either via KBr pelletization or using an attenuated total reflectance (ATR) setup, within a spectral range of 4000–500 cm^{-1}. Characteristic vibrational bands corresponding to hydroxyl groups, glycosidic bonds, and other structural features were recorded. All spectroscopic analyses were conducted under standardized conditions to ensure consistency **[45]**.

3. Results

3.1. Isolation of lactic acid bacteria

All seven fermented cucumber samples yielded observable bacterial growth, indicating the presence of diverse microbial communities, including lactic acid bacteria (LAB). The recovery and growth intensity of LAB varied among the isolates, reflecting differences in microbial abundance and adaptability. Notably, isolates L3 and L5 demonstrated the most robust growth on MRS medium under the tested conditions.

3.2. Purification of bacteria

The initial enrichment process, conducted at 37°C with continuous agitation, led to robust LAB proliferation; the stepwise sub-culturing strategy resulted in an increased concentration of viable LAB strains. Additionally, clear morphological differences were observed between isolates, indicating the presence of a diverse LAB community across the samples.

Table1. Growth Performance of Lactic Acid Bacteria Isolates Obtained from Fermented Cucumber

Isolates	Growth on MRS Agar	Relative Growth Intensity
L1	+	Moderate
L2	+	Moderate
L3	+++	High
L4	++	Moderate–High
L5	+++	High
L6	++	Moderate–High
L7	+	Moderate

3.3 Identification of lactic acid bacterial isolates

Microscopic examination revealed that all lactic acid bacteria (LAB) isolates were Gram positive. All isolates were non-motile.

Biochemical profiling of the isolates was conducted to further confirm their identity. All isolates tested catalase-negative, as evidenced by the absence of bubble formation upon exposure to 3% hydrogen peroxide, indicating the lack of catalase enzyme. The oxidase test also yielded negative results for all strains; no purple coloration developed within 30 seconds, confirming the absence of cytochrome c oxidase. Similarly, the coagulase test showed that none of the isolates induced clot formation in rabbit plasma, confirming their coagulase-negative phenotype, aligning with typical traits of *Lactobacillus* ssp.

Table2. Biochemical characterization of *Lactobacillus* ssp. isolates

Isolates	Catalase Test	Oxidase Test	Coagulase Test	Glucose Ferment-ation	Lactose Fermen -tation	Sucrose Fermen -tation	Maltose Ferment-ation
L1	Positive	Negative	Negative	Positive	Positive	Positive	Positive
L2	Positive	Negative	Negative	Positive	Positive	Positive	Positive
L3	Positive	Negative	Negative	Positive	Positive	Positive	Positive
L4	Positive	Negative	Negative	Positive	Positive	Positive	Positive
L5	Positive	Negative	Negative	Positive	Positive	Positive	Positive
L6	Positive	Negative	Negative	Positive	Positive	Positive	Positive
L7	Positive	Negative	Negative	Positive	Positive	Positive	Positive

3.5 Viscosity Assay to Dextran Production

The cultures were incubated at 37°C for 24 to 48 hours, under aerobic conditions. Following incubation, each broth culture was visually inspected for changes in physical consistency. The presence of increased viscosity manifested by stringy, thick, or slimy appearances was interpreted as a qualitative indication of extracellular dextran production.

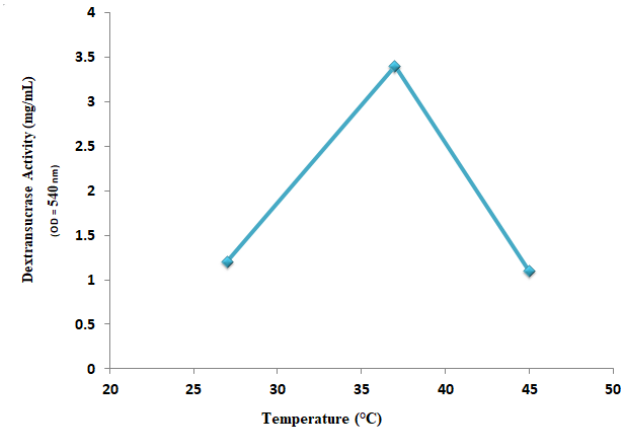
3.5. Determination of dextransucrase enzyme activity

Dextransucrase activity for the seven LAB strains is summarized in Table 3 & Figure1. Activity ranged from 0.0139 to 0.0377 U/mL. Highest activity was in isolate L5 (*Lactobacillus* sp. HH2; 0.0377 U/mL), while lowest in isolate L1 (0.0139 U/mL). Results again confirm that dextransucrase secretion is strain-specific, and indicate HH2 as the most promising isolate for dextran production.

Table3. Dextransucrase activity of LAB isolates obtained from fermented

Isolates	L1	L2	L3	L4	L5	L6	L7
Enzyme activity (U/mL)	0.0139	0.0189	0.0311	0.0328	0.0377	0.0178	0.0101

Figure1. Dextransucrase Activity of *Lactobacillus* spp. from



fermented cucumber

3.6 Optimization of Dextransucrase Activity

Lactobacillus ssp. HH2 isolate demonstrating the highest dextransucrase activity in preliminary screening (L5) was subjected to optimization studies. The effects of various physical and chemical parameters including temperature, incubation time, initial pH, sucrose concentration, and aeration were evaluated to identify the most favorable conditions for enzyme activity.

3.6.1 Effect of Temperature

Dextransucrase activity varied with incubation temperature. The highest activity was observed at 37 °C, while lower or higher temperatures led to reduced enzyme production. Notably, activity declined at both 27 °C and 45 °C, indicating a narrow optimum temperature range centered on 37 °C.

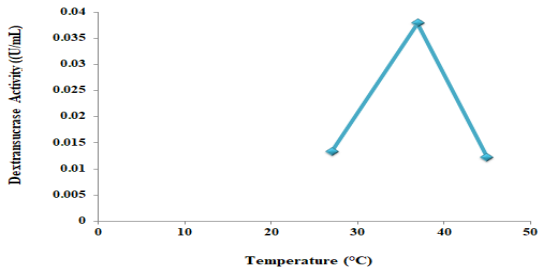


Figure2. Effect of Temperature on Dextransucrase Activity by *Lactobacillus* ssp. HH2

3.6.2 Effect of Incubation Time

Incubation time influenced enzyme output, with maximum activity recorded at 24 hours. Prolonged incubation (48 and 72 hours) resulted in a gradual decrease in dextransucrase activity, suggesting that extended culture duration may lead to enzymatic instability or nutrient depletion.

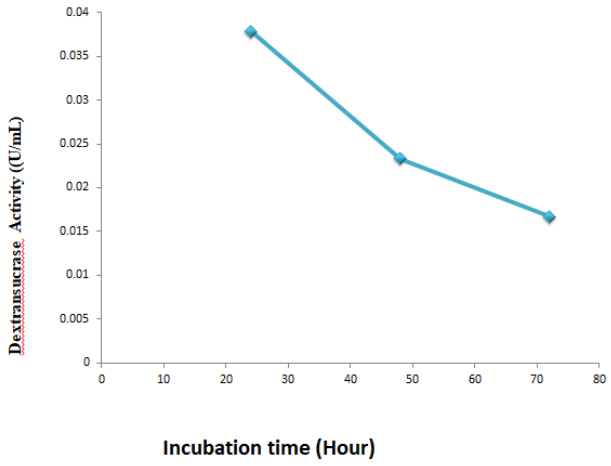


Figure3. Effect of Incubation Time on Dextransucrase Activity by *Lactobacillus* sp. HH2

3.6.3 Effect of PH

Enzyme activity was optimal at pH 6.0. Activity decreased under both acidic (pH 3.0–5.0) and alkaline (pH 9.0–11.0) conditions, indicating that near-neutral pH conditions are most suitable for dextransucrase functionality.

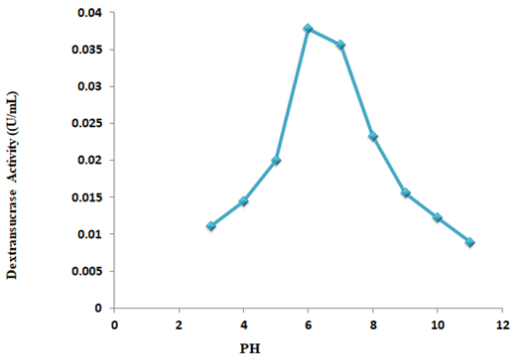


Figure4. Effect of PH on Dextransucrase Activity by *Lactobacillus* sp. HH2

3.6.4 Effect of Sucrose Concentration

Optimal enzyme activity was noted when the sucrose level was 10% (w/v). Low percentages (2–6%) gave suboptimal levels of activity, while elevated percentages above optimal (12% and 14%) did not give any increase in production, suggesting possible substrate inhibition when sugar is high.

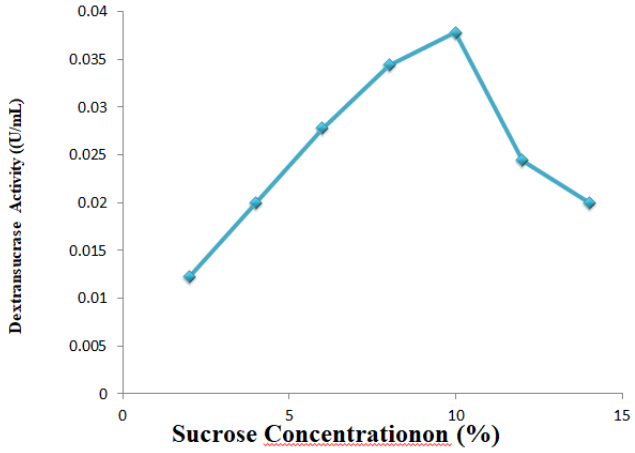


Figure 5. Effect of Sucrose Concentrationon DextransucraseActivity by *Lactobacillus sp*. HH2

3.6.5 Effect of Aeration

Dextransucrase activity was greater under aerobic conditions with mild agitation (50 rpm) compared to static anaerobic conditions. Oxygen availability appeared to support higher enzyme production, likely by facilitating better cell metabolism and protein synthesis.

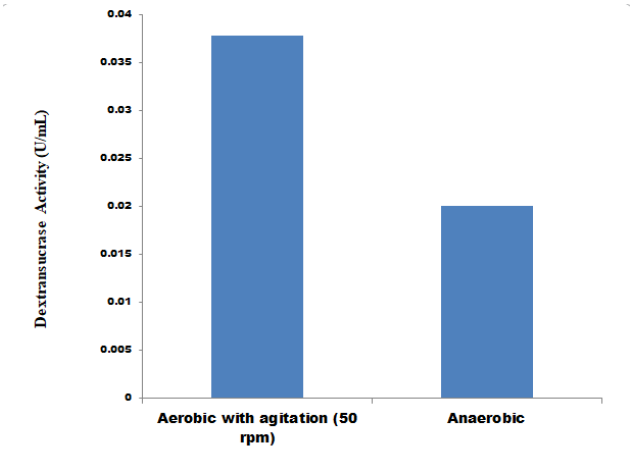


Figure6. Effect of Aeration on Dextransucrase Activity by *Lactobacillus* ssp. HH2.

3.7 Dextran Production by *Lactobacillus* sp. HH2

Dextran production by *Lactobacillus* sp. HH2 was successfully achieved using ethanol precipitation, a standard method for exopolysaccharide recovery. Crude culture supernatants underwent sequential ethanol precipitation steps to enhance polymer purity, followed by multiple washes with

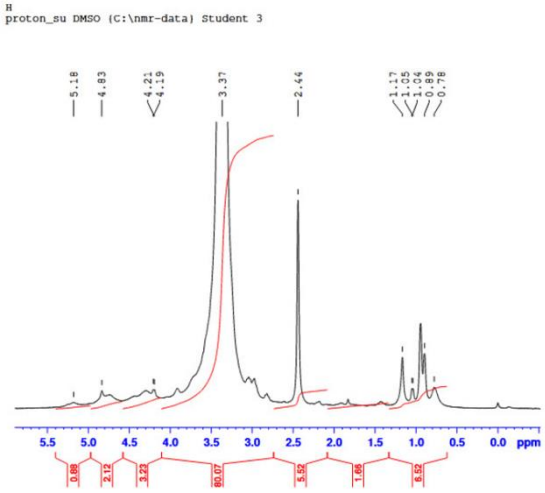
sterile distilled water. The resulting precipitate was subsequently lyophilized to obtain the final product. The purified dextran appeared as a dense, white pellet, which after lyophilization yielded a fine, dry powder. The powder displayed high water solubility, characteristic of hydrophilic polysaccharides. This physicochemical property is critical for its potential application in food stabilization, pharmaceutical formulations, and biomedical fields.

3.8 Structural Characterization of Dextran

3.8.1. Nuclear Magnetic Resonance (NMR) Spectroscopy Analysis

The ^{1}H NMR spectrum of the purified dextran sample in DMSO-d_6 confirmed its polysaccharide nature, predominantly composed of α-1,6-linked glucose units. A dominant peak at δ 3.37 ppm corresponds to ring protons (H-2 to H-6), while anomeric protons (H-1) appear in the δ 4.1–5.2 ppm range, indicating α-1,6 main chains with minor branching such as α-1,3 or α-1,2,6 linkages. A sharp singlet at δ 2.44 ppm is attributed to the DMSO solvent, and minor multiplets in the δ 0.78–1.17 ppm region may result from impurities or trace end groups.

Figure 7: ^{1}H NMR Spectrum of Dextran Produced by *Lactobacillus* ssp. HH2 in DMSO-d_6.



3.8.2. Fourier Transform Infrared (FT-IR) Spectroscopy Analysis

The Fourier Transform Infrared (FT-IR) analysis revealed distinct absorption bands characteristic of polysaccharides, supporting the identification of the extracted compound as dextran. A broad absorption band centered on 3400 cm^{-1} was observed, corresponding to O–H stretching vibrations—a common feature in hydroxyl-rich polysaccharides. Additionally, a sharp peak near 2920 cm^{-1} was attributed to C–H stretching vibrations, while a strong absorption region between 1150–1000 cm^{-1} was also evident, indicative of C–O–C and C–O–H stretching typically associated with glycosidic linkages. Figure 9 presents the FT-IR spectrum of the purified dextran extracted from *Lactobacillus* sp. HH2, where the broad O–H peak at 3400 cm^{-1}, the C–H stretch at 2920 cm^{-1}, and the intense bands in the fingerprint region collectively confirm the presence of α-glycosidic linkages, particularly α-(1→6) bonds, which are characteristic of dextran molecules. These spectral features confirm the carbohydrate nature of the biopolymer and strongly support that the isolated polymer is dextran.

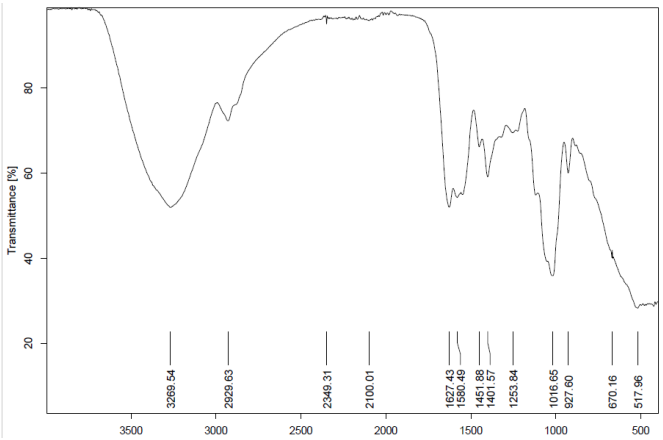


Figure 8: Fourier Transform Infrared (FT-IR) Spectral Analysis of Dextran Produced by *Lactobacillus* sp. HH2

4. Discussion

The successful isolation of *Lactobacillus* sp. HH2 from fermented cucumber highlights the adaptability of lactic acid bacteria (LAB) to diverse plant-based fermentation environments. Fermented cucumbers, rich in fermentable carbohydrates such as glucose and sucrose, create a selective niche that promotes the proliferation of acid-tolerant, non-motile, Gram-positive bacteria traits characteristic of LAB. The predominance of rod-shaped, catalase-negative, oxidase-negative, and coagulase-negative isolates observed in this study is consistent with well-established phenotypic features of the *Lactobacillus* genus, as documented in *Bergey's Manual of Systematic Bacteriology* and corroborated by previous studies on LAB biodiversity in fermented vegetables and dairy substrates **[46, 37, 47, 48, 49]**.The use of MRS medium for selective enrichment proved effective in recovering high-density LAB populations from fermented cucumber samples, reaffirming its utility as a gold-standard medium for LAB isolation from complex ecosystems **[50, 51]**. The biochemical profiles, particularly the uniform ability of all isolates to ferment glucose, lactose, sucrose, and maltose, further substantiated their metabolic alignment with dextran-producing LAB. Importantly, the non-motile phenotype observed across all isolates remains a key distinguishing feature of non-pathogenic, fermentative LAB **[11, 46]**.

Dextransucrase activity of *Lactobacillus* sp. HH2 was optimized near-neutrally and mesophilically with maximum yield at 37°C at pH 6–7 and 10% (wt/vol) sucrose. Activity was lowered at extreme pH values, sub-optimum incubation temperatures, and at higher sucrose levels due to substrate

inhibition. Production of the enzyme was at a maximum at 24 h and fell with longer incubation time, typical of growth-associated gene expression. Further, aerobic incubation with moderate agitation favoured activity relative to anaerobiosis and is used to underscore the role of oxidation and thus of oxygen transfer toward enzyme secretion. This result is consistent with recent findings pointing out availability of the carbon substrate, measurement of pH and controlled rates of aeration as main regulators of dextransucrase expression and dextran biosynthesis **[52, 53, 54].**

Dextran production by isolate HH2 was efficiently achieved through sequential ethanol precipitation, followed by lyophilization, yielding a white, water-soluble polysaccharide. The high solubility and hydrophilic nature of the purified dextran align with desired properties for biomedical and pharmaceutical applications, particularly as plasma volume expanders and drug delivery matrices **[27, , 55, 56]**. The recovery method used here is consistent with prior protocols that report recovery efficiencies exceeding 90% **[46]**.

Structural elucidation via FT-IR spectroscopy revealed characteristic spectral signatures of dextran. The broad absorption at ~3269 cm^{-1} indicated hydroxyl (O–H) stretching vibrations, while peaks at 2928 cm^{-1} and 2849 cm^{-1} were attributed to aliphatic C–H stretching. Strong bands at 1159, 1089, and 1018 cm^{-1} confirmed the presence of glycosidic (C–O–C) linkages, and a distinct absorption at 927 cm^{-1} was diagnostic of α-(1→6) glycosidic bonds—defining the polymer as a linear α-glucan **[54, 57, 58, 59]**.

Complementary ^1H NMR analysis further confirmed this structural identity. A dominant signal at 3.37 ppm was attributed to protons in α-(1→6)-linked glucose residues, with additional downfield peaks near 5.18 ppm and 4.23–4.93 ppm corresponding to anomeric protons and minor α-(1→3) and α-(1→2,6) branches. These findings match well with earlier NMR-based studies on microbial dextran structures **[22, 52]**. Together, the FT-IR and NMR results confirm that the produced polymer is a dextran composed primarily of α-(1→6) linkages with limited branching a configuration ideal for achieving desirable rheological and osmotic properties in biomedical contexts **[36]**.

The suitability of dextran from *Lactobacillus* sp. HH2 for medical applications is further reinforced by its high water solubility, potential biocompatibility, and non-pathogenic microbial source. Compared to plasma-derived volume expanders, microbial dextran presents a lower risk of immunogenicity and pathogen transmission, supporting its use in ethically favorable and scalable bioproduction systems **[59] [60]**.

This study confirms that fermented cucumber is not only a viable but also a highly effective substrate for isolating dextran producing LAB. The physicochemical characteristics of the extracted dextran, particularly its solubility and branching structure, highlight its promise for use in plasma substitution therapy, drug encapsulation, and possibly tissue scaffolding applications.

5. Conclusion

This study successfully established the potential of *Lactobacillus sp*. HH2, isolated from fermented cucumber, as a robust microbial source for high-quality dextran production. Under optimized conditions (37 °C, pH 6.0, 10% sucrose, 24 h incubation, and aerobic agitation), this strain demonstrated the highest dextransucrase activity among the tested isolates, underscoring its capacity for efficient exopolysaccharide biosynthesis.

The dextran was recovered through sequential ethanol precipitation and lyophilization, yielding a white, highly water-soluble polymer. Structural characterization using FT-IR and ^1H NMR spectroscopy confirmed the presence of predominantly α-(1→6)-linked glucose residues, with minor α-(1→3) and α-(1→2, 6) branching. These structural features are characteristic of pharmaceutical-grade dextran and are directly associated with favorable rheological and osmotic properties for biomedical use.

Importantly, the dextran produced by *Lactobacillus* sp. HH2 demonstrated key physicochemical traits; solubility, biocompatibility, and defined branching that support its application as a plasma volume expander and as a carrier matrix for drug delivery systems. The use of a non-pathogenic, food-grade LAB strain further enhances the safety, scalability, and regulatory acceptance of this production method.

Overall, this research highlights the viability of LAB-based dextran biosynthesis as a sustainable approach for medical aplication and food industries. Fermented cucumber was identified as a valuable, underutilized reservoir of biofunctional LAB, reinforcing the potential of plant-based matrices in microbial biotechnology.

Future studies should focus on scaling up production using bioreactors, optimizing fermentation dynamics and tailoring the molecular weight and branching architecture of the polymer to meet the requirements of targeted clinical and pharmaceutical applications, including parenteral therapies and tissue engineering.

CRediT authorship contribution statement:

Conceptualization, A.E, M.M and M.A. designed the study; H.H. performed the experiments and A.E contributed to the writing. All authors have read and agreed to the published version of the manuscript.

Data availability statement

The data used to support the findings of this study are available from the corresponding author upon request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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