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PRODUCTION AND CHARACTERIZATION OF DEXTRAN BIOSYNTHESIZING GLUCOSYLTRANSFERASE FROM *L. MESENETEROIDES* KIBGE-IB40

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ABSTRACT

Exploration of biodiversity led towards the development of commercially important biological metabolites. This study focus on the molecular characterization of dextransucrase biosynthezing lactic acid bacterial strain, *L. mesenteroides* KIBGE-IB40. Fermentation system was designed to optimize the crucial production parameters of the biosynthesis of dextransucrase by *L. mesenteroides*. Data acquired from the experimental analysis revealed a significant increase of about 1.69-fold in enzyme titer from 67.13 DSU/ml to 114.04 DSU/ml. The optimum fermentation conditions were found to be fermentation time, 18 hours; fermentation temperature, 25°C; fermentation pH, 7.5. Structural analysis of purified dextran was performed. The morphological analysis of dextran by SEM revealed a porous structure of the polysaccharide. FTIR analysis demonstrates the functional group analysis of the polymer. The significant increase in dextransucrase yield demonstrates the crucial impact of fermentation conditions on the metabolic properties of microbial cells. The obtained results demonstrate a practical approach for the commercial application of *L. mesenteroides* KIBGE-IB40 dextransucrase in food and other industrial sectors.

Keywords: Biopolymer; Dextransucrase; Dextran; Fermentation; *Leuconostoc mesenteroides*; Optimization

INTRODUCTION

Dextran is a homopolysaccharide of glucose molecule, exhibiting contiguous Cα-1 → Cα-6 glycosidic bond in the main chain with different types of branches including Cα-1 → Cα-2, Cα-1 → Cα-3, Cα-1 → Cα-4. It has been found to have significant application in food, feed, pharmaceutical and dairy industry. Properties such as biodegradability, biocompatibility, non-immunogenicity and non-toxicity are the attractive features which increases its applicability **(Takashima *et al.*, 2015).** Dextran and its derivatives have been used as promising biomaterial for nano-drug delivery system. Dextran enhances the stability of the drug delivery system and prevent it from accumulating into the blood system **(Ferrari *et al.*, 2021).** Dextran is used as a green corrosive inhibitor for steel in acid induced environment **(Solomon *et al.*, 2018)**. In baking, dextran is used as a hydrocolloid by altering the water binding and viscoelastic properties of gluten. Dextran improves the dough rheology, baking performance and enhances the flavor of bread **(Wang *et al.*, 2021)**.

The biosynthesis of dextran is catalyzed by a glucosyltransferase enzyme, dextransucrase (E.C. 2.4.1.5). The enzymatic mechanism involves hydrolysis of glycosidic bond present in sucrose molecule resulting in the transfer of glucopyranosyl residue to the growing dextran chain while, concomitantly releasing the fructose molecule. A variety of dextransucrase producing microbial species of different genus have been reported including *Leuconostoc*, *Weissella*, *Rhizophus*, *Lactobacillus*, *Oenocnoccus* and *Acetobacter* **(Azmi 2021; Vuillemin *et al.*, 2018; Sankpal *et al.*, 2001).** Dextransucrase produced from different bacterial strains differ in their physiochemical properties as well as applicability. The molecular size and branching pattern are characterized based on the producing microbial source. Dextransucrase produced from *L. mesenteroides* is most commonly used for commercial dextran production. Under suitable fermentations conditions, *L. mesenteroides* produces massive amount of dextransucrase. For any industrial fermentation process, its production parameters play a critical role on the overall cost of the process. These parameters increases the profit of the process by influences the formation, purity and yield of the product. Nevertheless, there is a need to establish new fermentation protocols as with every passing day the strains are mutated, and new strains are continuously explored. Different combinations of fermentation conditions are investigated to determine the best possible physiological state of microbial cells for metabolite production **(Schmidt 2005).** Therefore, the current study aims toward the molecular characterization of a dextransucrase producing *L. mesenteroides* strain. Furthermore, the fermentation conditions were optimized to improve the enzyme yield. Dextran produced under the optimized conditions was purified and the structural characteristics was studied.

MATERIALS AND METHODS

Chemicals

Yeast extract [LP0021] and Peptone [LP0037] were procured from Oxoid, Hampshire, England. Sucrose [35580], Dipotassium hydrogen phosphate [6887], Calcium chloride [15587], Sodium chloride [30184] were products of Serva, Heidelberg, Germany. Magnesium sulfate [MA0087] were of Scharlau, Barcelona, Spain. Double deionized water was used for the preparation of reagents.

Microbial Culture

The bacterial strain was isolated from fermented food samples. The isolate was morphologically and biochemically identified as *L. mesenteroides* KIBGE-IB40. The culture was maintained on tomato juice agar slant at 4°C and subcultured periodically. The inoculum was prepared by transferring the bacterial culture into the fermentation medium consisting of gL^{-1}: Sucrose 20.0, Yeast extract 5.0, Peptone 5.0, Dipotassium hydrogen phosphate 15.0, Calcium chloride 0.05, Sodium chloride 0.01, Magnesium sulphate 0.01, and Manganese chloride 0.01 with pH of 7.5. The culture was incubated at 25°C for 8h at 100 rpm **(Zafar *et al.*, 2019).**

Molecular Characterization

Genomic DNA was extracted for 16S rDNA sequence analysis using DNA extraction kit (Promega, USA). Amplification of the 16S rDNA was performed through polymerase chain reaction (PCR) using universal primers. The forward primer set was 5'-GAGTTTGATCCTGGCTCAG-3' whereas, the reverse primer was 3'-AGAAAGGAGGTGATCCAGCC-5'. Agarose gel (1%) electrophoresis was performed to analyze the purity of the DNA sample. The amplified PCR product was purified and sequenced. Phylogenetic tree was constructed using neighbor joining method (Mega software).

Optimization of fermentation parameters

Fermentation parameters exhibited critical role on the growth and biochemical activities of the microbial cell. The optimal fermentation conditions required for the maximum biosynthesis of dextransucrase was estimated. Dextransucrase activity was estimated by measuring the amount of reducing sugar using Nelson Somogi method **(Kobayashi and Matsuda, 1974).**

Fermentation time

Bacterial cell was incubated for 6 hours, 8 hours, 12 hours, 18hours, 24 hours, and 48 hours at 25°C.

Fermentation temperature

To determine the effect of fermentation temperature on the enzyme yield, the microbial strain was kept at different temperature including 20°C, 25°C, 30°C for constant time period and fermentation pH

Fermentation pH

The pH of the fermentation medium was optimized by adjusting the pH ranging from 6 to 8.

Dextran production and purification

Batch fermentation was performed under the optimized conditions for dextran production. The fermentation medium was mixed with equal volume of chilled ethanol for precipitation of dextran. The precipitates were centrifuged at 10,000 rpm for 15 minutes. The supernatant was decanted, and the pellet was dried over calcium chloride under vacuum at room temperature. The dried dextran was dissolved in distilled water and the slurry was mixed with chilled ethanol. This procedure was repeated thrice, and the purified dextran was dried **(Sarwat *et al*., 2008).**

Scanning electron microscopy

The purified sample of dextran was bound on to the SEM stub with double sided tape and encrusted with gold particles at 30Å in a Quick Auto Coater (JFC-1500 JEOL). The coated sample was analyzed using SEM (JEOL, Japan). The images were observed under different magnification power at 10.0 kV.

Fourier transforms infrared spectrophotometry

The FTIR analysis of purified dextran was recorded using FTIR coupled with ATR accessory (Thermo Nicolet™ iS™ 5 FTIR spectrophotometer). Dried sample was powdered and placed inside the ATR chamber equipped with Zinc selenide (ZnSe) crystal. The spectrum was scanned at wave number ranges between 4000 cm^{-1} and 550 cm^{-1}in the transmittance mode with a number of scans of 5. The infrared spectrum resolution was noted at 4 cm^{-1}. The peaks of dextran were recorded and analyzed using OMNIC software.

RESULTS

Molecular characterization and phylogenetic analysis

The 16S rDNA genome analysis of *L. mesenteroides* KIBGE-IB40 was performed. A single band of genomic DNA was observed which confirms the homogeneity of the DNA.

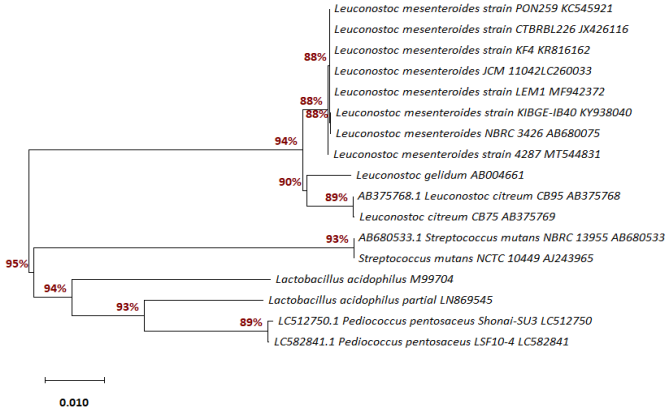

Figure 1 Phylogenetic relationship of *L. mesenteroides* KIBGE-IB40 with other bacterial strains

The purified sample was subjected to PCR amplification which revealed a distinct 1600 bp band of the amplicon. Sequence similarity was analyzed, and the sequence get the NCBI accession number of KY938040. Phylogenetic tree was constructed to infer the relatedness of *L. mesenteroides* with other bacterial strains. *L. mesenteroides* KIBGE-IB40 share high relatedness to the other identified *L. mesenteroides* than other genus and species of lactic acid bacteria (Figure 1).

Optimization of fermentation parameters for enhanced biosynthesis of dextransucrase

In the current study, fermentation parameters were optimized using conventional *one-factor-at-a-time* (OFAT) approach.

Fermentation time

Firstly, fermentation time was optimized to provide favorable environmental conditions to the bacterial isolate for their maximum growth and enzyme production. The results revealed that the microbial cells started to divide after 06 hours and reached maximum growth during the 08 hours of fermentation. After that, bacteria enter into the stationary phase and the growth remains constant till 48 hours. The pattern for enzyme biosynthesis was also similar, maximum enzyme production was noted during the 08 hours of exponential growth phase of bacteria. Therefore, it can be stated that bacterial growth is associated with the enzyme production and 08 is considered as optimum fermentation time for dextransucrase biosynthesis from *L. mesenteroides* KIBGE-IB40 (Figure 2).

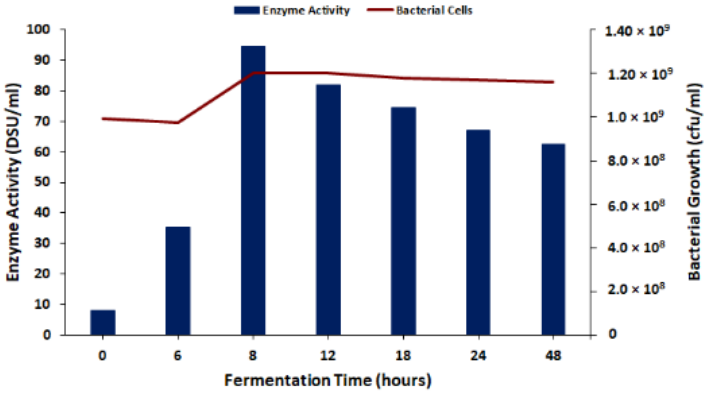

Figure 2 Effect of fermentation time on dextransucrase biosynthesis

Fermentation temperature

The optimum fermentation temperature of dextransucrase biosynthesis was determined by keeping the bacterial isolates at different temperatures ranges from 20°C to 30°C for constant time interval and pH. The results revealed that maximum metabolic growth and activity of bacteria was noticed at 25°C after that, both the multiplication of microbial cells and enzyme production was decreased (Figure 3).

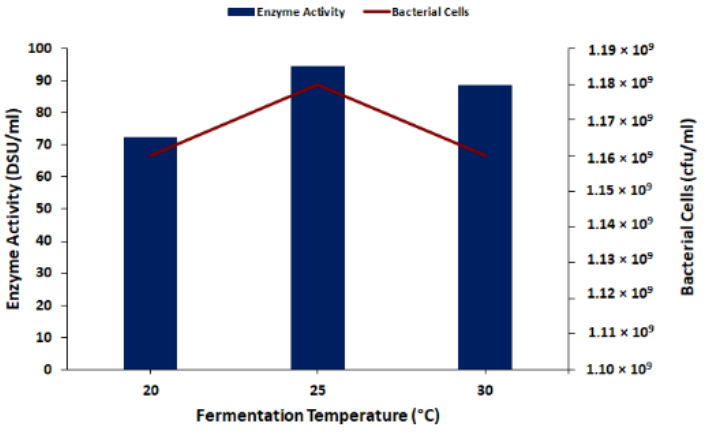

Figure 3 Effect of fermentation temperature on dextransucrase biosynthesis
Fermentation pH

The optimum pH for dextransucrase produced from *L. mesenetroides* KIBGE-IB40 was investigated at constant fermentation time and temperature of 8 hours and 25°C, respectively (Figure 4). The results revealed that *L. mesenteroides* KIBGE-IB40 exhibited both the maximum metabolic growth and enzyme yield at pH 7.5.

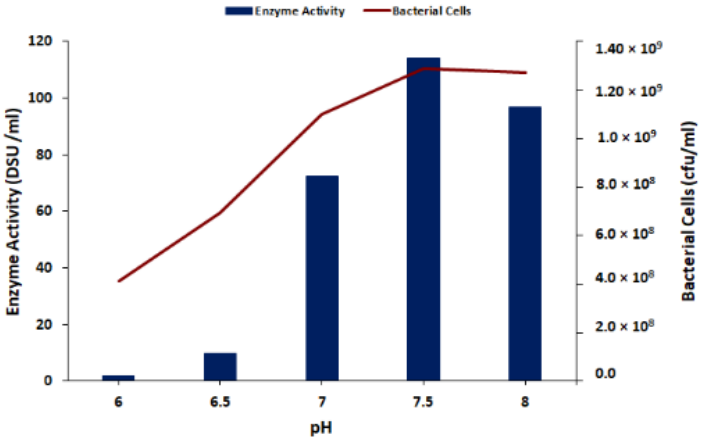

Figure 4 Effect of fermentation pH on dextransucrase biosynthesis

Surface morphological studies of dextran

Surface topology of dextran was observed at different resolution using SEM (Figure 5). A highly compact structure with small pores was observed which exhibits the high-water retention ability of dextran.

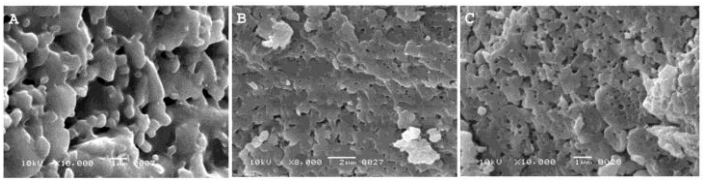

Figure 5 SEM showing surface morphology of sucrose (A) and dextran at different magnification levels 8, 000 X (B) and 10,000 X (C)

FT-IR analysis

FTIR spectroscopy was performed to determine the functional group of dextran (Figure 6). The FTIR spectrum indicate that the polysaccharide contains hydroxyl group by exhibiting absorption peak around 3400 cm^{-1} (3200 cm^{-1} to 3600 cm^{-1}) **(Wang *et al*., 2010).** The band at 2921 cm^{-1} was related to the C-H bond of the polysaccharide and the peak at 1647 cm^{-1}indicates the existence of C=O bond **(Ye *et al*., 2014).** The band in the region of 1559 cm^{-1} was possibly due to the N-H bending of amide-II of protein **(Lin *et al*., 2005).** The absorption peak at 1416 cm^{-1} was due to stretching vibration of C-O bond (**Zhou *et al*., 2018)**. The peak at 1155 cm^{-1}might be assigned to C-O-C and glycosidic bridge. The stretching band around 1015 was due to the presence of α-1,6 glycosidic linkage **(Shingel, 2002)**. The band at region of 915 (600-950 cm^{-1}) was due to the pyranose form of glucosyl residue **(Kavitake *et al*., 2016)**.

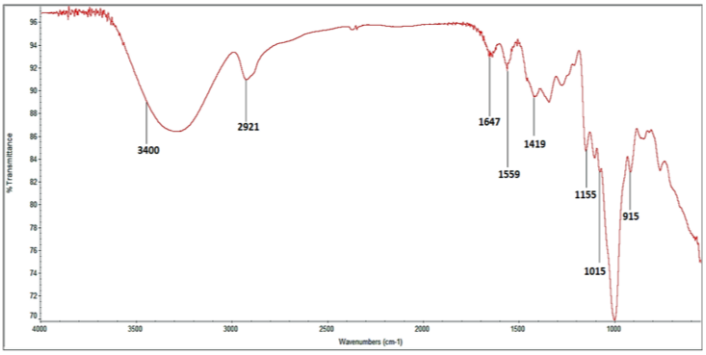

Figure 6 FTIR spectrum of dextran produced from *L. meseneteroides* KIBGE-IB40

DISCUSSION

Microbial fermentation is a complex biotechnological process through which bioactive compounds can be produced. Minor variation in the medium components and culturing environment leads a variation in the metabolic profile of the microbial strain. The change in the metabolic activities of the microbial cells also influences the yield of the product. In any bioprocessing, a large amount of cost is attributed to the designing of an experiment. For this purpose, various strategies have been reported to optimize the fermentation parameters for dextransucrase production **(Miljković *et al*., 2021; Rahman *et al*., 2020).** Conventional method is the most simplified and commonly used approach used for the optimization processes, thereby promoting the discovery of novel microbial compounds. Firstly, fermentation time was optimized. Maximum microbial multiplication was noted during the log phase of bacterial profile. Decline in bacterial growth after the log phase might be due to the depletion of essential nutrients required for microbial cultivation. Secondary metabolites produce during the stationary phase also inhibit the metabolic activities of the microbial cells. Maximum dextransucrase yield was also noted during that period (8 hours) of the bacterial growth. In another study, *L. mesenteroides* AA1 also produces maximum dextransucrase at 08 hours of fermentation period **(Aman *et al*., 2012)**. Dextransucrase from *L. mesenteroides* B/110-1-1 achieved maximum growth rate and enzyme titer between 6 to 7 hours of fermentation **(Michelena *et al*., 2003)**. Optimum temperature was also observed by incubating the microbes at different temperatures (20°C, 25°C and 30°C). Change in environmental condition affect the fluidity of the cytoplasmic membrane, which resulted in the fluctuation of membrane permeability. This change in permeability affects the transport of important components across the membrane. Also, it allows the entry of potentially harmful substances which can inactivates the catalytically important proteins **(Beney and Gervais, 2001)**. The optimum fermentation temperature for *L. mesenteroides* KIBGE-IB40 dextransucrase was 25°C. Previously, similar results were also noted for dextransucrase production from different genus of lactic acid bacteria. *W. confusa* Cab3 which exhibited maximum enzyme biosynthesis at 25°C during fermentation time of 12 to 15 hours with pH of 7.0 **(Cortezi *et al.*, 2005)**. *L. meseneteroides* NRRL B 512 F and *L. meseneteroides* FT 045 B produces dextransucrase yield of 49.3 and 3.2 DSU ml^{-1} at temperature of 23 to 25°C, respectively **(Shukla and Goyal, 2011).** Dextransucrase from *L. mesenteroides* T3 was observed at 23°C for 12 hours under shaking of 180 rpm **(Miljković *et al*., 2021).** Microbes are sensitive to the surrounding environmental conditions. The pH of the medium affects the structural integrity of the proteins or enzymes. Any fluctuation in the medium pH changes the nature of the protein by altering the ionization potential of the functional groups and the disruption of the interaction between the polypeptide chains of the protein molecule. This change in pH modifies the protein secondary and tertiary structure which ultimately alters the catalytic properties of the enzyme **(Damodaran, 2008).** The obtained data of the current study demonstrates the neutrophilic nature of the bacterium. Similar pattern of results was also noted for dextransucrase produced from *L. mesenteroides* KIBGE-IB22 and *L. mesenteroides* EA6 **(Siddiqui *et al*., 2013; Qader *et al*., 2007)**.

Morphological studies of dextran produced by *L. mesenteroides* KIBGE-IB40 dextransucrase was analyzed. SEM demonstrates the porous water-soluble nature of the polymer. The porous structure improves the physical properties e.g.,thickening, stabilizing, emulsifying of food products by forming a hydrated polymer consistent matrix. The porous structure of dextran provides compactness and stability to the polymer against environmental conditions and promotes the applicability of dextran in food and cosmetic industries **(Wang *et al*., 2021; Wang *et al*., 2019)**. Porous structure in this study was similar to dextran produced from *L. meseneteroides* KIBGE-IB22 **(Siddiqui et al., 2014),** *L. kimchi* **(Torres-Rodríguez *et al*., 2014)**, part structure of dextran from *Leuconostoc pseudomesenteroides* XG5 which exhibit porous and highly branched surface **(Ahmed *et al*., 2013),** but different from polymer produced by *L. citreum* NM105 which showed a highly branched and glittering surface **(Yang *et al*., 2015),** and *L. mesenteroides* NRRL B-1149 which exhibit a cubical porous structure **(Shukla *et al*., 2011)**. The extent of intermolecular interaction between the polymer matrixes was investigated by FTIR. The data obtained from the current study exhibited the major characteristic peaks of dextran reported previously **(Rosca *et al*., 2018; Siddiqui *et al*., 2014).** The alterations in the morphology and the microstructure of the polymer might be due to the difference in the composition and structure of the monosaccharide produced by diverse microbial strains.

CONCLUSION

In this study, a dextran producing strain of lactic acid bacteria isolated from fermented food product was identified as *L. meseneteroides* KIBGE-IB40. Fermentation model was established to maximize the biosynthesis of dextransucrase. Surface morphology revealed a highly porous structure of dextran that makes it a promising candidate for exploitation in food industry. FTIR spectrum revealed the functional group pattern of the polymer. In conclusion, it is important to explore dextran producing novel microorganisms and study their structural characteristics. This will increase the commercial applicability of the polymer.

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