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# Mutational analysis and characterization of dextran synthesizing enzyme from wild and mutant strain of *Leuconostoc mesenteroides*

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ABSTRACT

Dextransucrase producing *Leuconostoc mesenteroides* KIBGE IB-22 was subjected to mutagenesis by exposing the strain to UV irradiation. The dextransucrase produced by both the strains (wild and mutant) were characterized and the catalytic properties of both wild and mutant dextransucrase were compared. Among 42 mutants, KIBGE IB-22M20 exhibited 6.75 times increase in dextransucrase activity as compared to the wild one. Wild dextransucrase showed specific activity of 31.3 DSU/mg of protein with $V_{max}$ and $K_m$ of 18.84 DSU/ml/h and 77.09 mM, respectively at 30 °C in 0.3 M citrate buffer (pH 4.5) using sucrose as substrate. Whereas, mutant dextransucrase exhibited a specific activity of 173.2 DSU/mg with $V_{max}$ and $K_m$ values of 104.2 DSU/ml/h and 101.7 mM, respectively at 35 °C in 0.3 M citrate buffer (pH 5.0) keeping the same substrate as for wild. Dextransucrase from both wild and mutant showed an approximate molecular weight of 221 kDa by SDS-PAGE.

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## 1. Introduction

Dextran is a commercial biopolymer produced by some of the selected strains of lactic acid bacteria. This important polysaccharide has significant commercial value with reference to its molecular weight (Aman, Siddiqui, & Qader, 2012; Kim & Day, 1994; Leathers, Hayman, & Cote, 1995; Shamala & Prasad, 1995). The formation of this biopolymer is dependent upon a glucosyltransferase, belonging to glycoside hydrolases family (GH70) and is known as dextransucrase (2.4.1.5) (Bounaix et al., 2010; Henrissat & Davies, 1997). This inducible extracellular enzyme catalyzes the synthesis of high molecular weight dextran from sucrose as well as low molecular weight oligosaccharide in the presence of maltose and isomaltose (Koepsell et al., 1953; Sidebotham, 1974). Nowadays, high production of dextransucrase and dextran is of prime importance for industrial purposes. Therefore, improvement of strain for enhanced production of industrially important enzyme is an important feature of mutagenesis and this can be achieved by exposing the natural isolate to different mutagenic agents.

Numerous effective mutagenic procedures including physical, chemical and site directed mutagenesis have been reported for the improvement of strains. Sometimes a combination of two mutagenic agents is also used for a reliable mutation purpose. Several studies have been conducted in order to increase the production of dextransucrase and dextran from various lactic acid bacteria (LAB) by using different mutagenic agents including UV radiation (Kamal, Samadi, Mazaheri, Moazami, & Fazeli, 2001; Kothari, Tyagi, Patel, & Goyal, 2011; Patel & Goyal, 2010), ethyl methanesulfonate (Kim & Robyt, 1994, 1995; Smith, Zahnley, & Goodman, 1994) and N-methyl-N′-nitro-N-nitrosoguanidine (Kitaoka & Robyt, 1998; Smith & Zahnley, 1997). All the mutants acquired were screened for over-expression of dextransucrase and rarely any study discusses the kinetic expression of this enzyme produced after mutagenesis.

Current study deals with an attempt to improve the production of dextransucrase from *Leuconostoc mesenteroides* KIBGE IB-22 (wild) through mutagenesis and to characterize the enzyme form wild and the mutant strains. *L. mesenteroides* KIBGE IB-22 was exposed to UV irradiation and the mutants obtained were initially screened for over-expression of dextransucrase and than characterized on the basis of catalytic properties with reference to natural isolate. This study on the kinetic expression of dextransucrase from wild and mutant strain will provide important information for industrial uses of enzyme.

## 2. Materials and methods

### 2.1. Isolation and identification of L. mesenteroides

Ten different strains of *L. mesenteroides* were isolated from various fermented plant materials including bitter gourd (*Momordica charantia*), lady finger (*Hibiscus esculentus*), carrot (*Daucus carota*), cauliflower (*Brassica oleracea* var. *botrytis*), tomato (*Lycopersicon esculentum*), persimmon (*Diospyros kaki*), ridge gourd (*Luffa acutangula*) and were designated as KIBGE IB-6, KIBGE IB-7, KIBGE IB-8,

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KIBGE IB-9, KIBGE IB-11, KIBGE IB-12 and KIBGE IB-13, respectively. Whereas, two strains were isolated from cabbage (*Brassica oleracea* var. *capitata*) and labeled as KIBGE IB-10 and KIBGE IB-22. One more strain was isolated from molasses that was obtained from the local sugar industry and designated as KIBGE IB-19.

From each plant material 5.0 g sample was inoculated into 10.0 ml of selective medium which contained (g/l): sucrose, 100; yeast extract, 5.0; tryptone, 5.0; $K_2HPO_4$, 1.0 and sodium azide, 1.0. The pH of the medium was adjusted to 7.5 and was autoclaved at 121 °C for 15 min. The medium containing different samples was kept at 25 °C for 24–48 h. A loop full of culture from these tubes after fermentation were streaked on the selective medium agar plates and again incubated at 25 °C for 24 h. After 24 h, selection of specific slime producing colonies from each sample plate was made and the identification of the strains was performed on the basis of morphological, biochemical and molecular analysis. Bergy's manual of determinative bacteriology was used for the identification of bacterial cultures on the basis of morphological and biochemical parameters (Holt, 1994) whereas, 16S rDNA sequence analysis were performed for molecular analysis. The purified stock cultures were preserved on tomato juice agar slants at 4 °C (Aman et al., 2012).

DNA was extracted for 16S rDNA sequence analysis by the method described earlier (Chen & Kuo, 1993). The conditions for PCR and the universal primers used for the amplification of 16S rDNA fragment were same as reported (Ansari, Aman, Siddiqui, Iqbal, & Qader, 2012).

### 2.2. Sequence analysis

Sequence similarity searches (Blast) for the current strains of *L. mesenteroides* were performed by comparing the sequence of 16S rDNA available in GenBank database (http://www.ncbi.nlm.nih.gov/BLAST/). Final sequences of 10 isolates were submitted to the EMBL database (http://www.ebi.ac.uk/embl). Multiple sequence alignments were performed using Clustal X (Thompson, Gibson, Plewniak, Jeanmougin, & Higgins, 1997). Phylogenetic trees were constructed using the neighbor joining algorithm of Clustal X and displayed, manipulated and printed using Treeview (Page, 1996). The sequences from *L. mesenteroides* KIBGE IB-22 (wild) and *L. mesenteroides* KIBGE IB-22M (mutant) were aligned and MegAlign program of Lasergene (DNA Star Inc., Madison, WI, USA) was used to determine the percentage identity figures by assembling and analyzing the sequences using the software. Alignment was conducted using the Clustal V algorithm.

### 2.3. Nucleotide sequence accession numbers

Sequence reported in this study have been deposited in the GenBank with following accession numbers, *L. mesenteroides* KIBGE IB-6 [GenBank: GU907668], *L. mesenteroides* KIBGE IB-7 [GenBank: GU907669], *L. mesenteroides* KIBGE IB-8 [GenBank: GU907670], *L. mesenteroides* KIBGE IB-9 [GenBank: GU907671], *L. mesenteroides* KIBGE IB-10 [GenBank: GU907672], *L. mesenteroides* KIBGE IB-11 [GenBank: GU907673], *L. mesenteroides* KIBGE IB-12 [GenBank: GU907674], *L. mesenteroides* KIBGE IB-13 [GenBank: GU907675], *L. mesenteroides* KIBGE IB-19 [GenBank: HQ588348], *L. mesenteroides* KIBGE IB-22 [GenBank: JQ658345], *L. mesenteroides* KIBGE IB-22M [GenBank: JQ658346].

### 2.4. Medium composition

Dextransucrase was produced by batch fermentation and the defined enzyme production medium contained (g/l): sucrose, 20.0; yeast extract, 5.0; tryptone, 5.0; $K_2HPO_4$, 15.0; NaCl, 0.01; $MnCl_2$, 0.01; $MgSO_4 \cdot 7H_2O$, 0.01; $CaCl_2$, 0.05 and the pH was maintained at 7.5 (Qader, Iqbal, Aman, Shireen, & Azhar, 2005). Inoculum (10.0 ml) was prepared at 25 °C for 24 h. For the preparation of seed culture the inoculum was transferred into 90.0 ml medium and was incubated for 24 h at 25 °C. This seed culture was then transferred into 900.0 ml medium and incubated at 25 °C for 18 h under static condition. After harvesting the cells by centrifugation at 35,000 × *g* for 15 min at 4 °C, the cell free supernatant was separated and was used for further studies.

### 2.5. Enzyme assay and protein determination

The dextransucrase activity assay was performed according to the method as describe by Kobayashi and Matsuda (1974). Enzyme (50.0 μl) was incubated with 1.0 ml sucrose (125 mg/ml) prepared in 0.1 M citrate phosphate buffer (pH 5.0) at 35 °C for 10 min. After incubation the reaction was stopped by adding 1.0 N NaOH (50.0 μl). Units of dextransucrase activity are represented as DSU/ml/h. One unit of enzyme activity is defined as "The amount of enzyme that converts 1.0 mg of sucrose into fructose and dextran under standard assay conditions" (Lopez & Monsan, 1980).

Total protein concentrations of the samples were calculated using bovine serum albumin as standard (Lowry, Rosebrough, Farr, & Randall, 1951).

### 2.6. Mutagenesis using UV irradiation of L. mesenteroides (wild type)

*L. mesenteroides* KIBGE IB-22 (wild) was subjected to UV irradiation and was grown in medium containing (g/l): glucose, 10.0; yeast extract, 5.0; tryptone, 5.0; $K_2HPO_4$, 15.0; NaCl, 0.01; $MnCl_2$, 0.01; $MgSO_4 \cdot 7H_2O$, 0.01 and $CaCl_2$, 0.05. The pH of the medium was adjusted to 7.5 and was autoclaved at 115 °C, 15 lb pressure for 20 min. The liquid medium in the flask was inoculated with a loop full culture of growing *L. mesenteroides* KIBGE IB-22 (wild) and was incubated at 25 °C for 12 h with shaking at 150 rpm. The optical density (OD) of the grown culture was measured using spectrophotometer at 600 nm. For mutagenesis the cells were aseptically harvested from the log phase of the growing culture at 35,000 × *g* for 15 min at 4 °C. Supernatant was discarded and the cell pellet was re-suspended in sterilized normal saline. The initial OD of cell suspension was adjusted to 0.4 by adding sterilized normal saline. One milliliter of the resulted cell suspension was transferred to 9.0 ml of sterile saline and serial dilutions of $10^{-1}$, $10^{-2}$, $10^{-3}$, $10^{-4}$, and $10^{-5}$ were prepared. Two parallel sets, one for control (wild) and other for exposure to UV radiation were prepared by spreading 100.0 μl cell suspension from each dilution, *i.e.* $10^{-3}$, $10^{-4}$, and $10^{-5}$ on glucose agar medium plates. Control sets of each dilution (wild) were incubated at 25 °C for 24 h without exposure to UV irradiation. To generate mutants, second set of spread plates from each dilution, *i.e.* $10^{-3}$, $10^{-4}$, and $10^{-5}$ were exposed to UV radiation (15 W) from distance of 25.0 cm for different time intervals (05, 10, 15, 30 and 60 s). After UV exposure the plates were kept in dark for 02 h and then incubated at 25 °C for 24–48 h. The radiation dose was chosen to give at least 0.1% survivors. For screening of dextransucrase activity morphologically similar colonies showing slimy shiny texture were picked from each set of control and UV exposed plates and were incubated in enzyme production medium for 24 h at 25 °C. The mutant that showed the highest enzyme activity as compared to parent strain (wild) was selected for subsequent experiments.

### 2.7. Polyethylene glycol fractionation

After cell harvesting the crude enzyme was subjected to partial purification using polyethylene glycol-4000 (PEG-4000). In 1.0 l of cell free supernatant, saturated $CaCl_2$ (0.1 g/dl, v/v) was added.

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