Carbohydrate Polymers 91 (2013) 209–216

Contents lists available at SciVerse ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

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

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ARTICLE INFO

Article history:
Received 26 June 2012
Received in revised form 21 July 2012
Accepted 8 August 2012
Available online 16 August 2012

Keywords:
Dextransucrase
Mutation
UV irradiation
Dextran
Leuconostoc mesenteroides

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.

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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http://dx.doi.org/10.1016/j.carbpol.2012.08.026

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.

Chilled PEG-4000 solution (30.0 g/dl, w/v) was incorporated up to 25.0% saturation with continuous stirring and was left for equilibration for 18 h at 4 °C. The protein precipitates formed from wild and mutant strains were centrifuged at 35,000 × *g* at 4 °C and were resuspended in 0.3 M citrate buffer of pH 4.5 and 5.0, respectively. The partially purified dextransucrase was then used for further studies.

2.8. Kinetic studies of extracellular dextransucrase from wild and mutant strains

Kinetic behavior of extracellular dextransucrase from wild and mutant of *L. mesenteroides* were analyzed.

(a) Selection of buffer, pH and ionic strengthInitially four different buffers, *i.e.* sodium phosphate, citrate, acetate, and citrate phosphate buffers were used to confirm the suitable buffer for maximum dextransucrase activity. The strength and pH of all buffers were kept at 0.1 M and 5.0, respectively. After selection of the buffer for both the wild and mutant strains the pH of the buffer was varied from 3.5 to 7.5 by an increment of 0.5. Further, ionic strength of the selected buffer was varied from 0.05 to 0.3 M concentration.
(b) Reaction time, substrate kinetic and temperature maximaMaximum extracellular dextransucrase activity was determined by incubating the enzyme with substrate (sucrose) at 35 °C. Activities were calculated at different time intervals (10, 15, 30, 45 and 60 min). To study the influence of substrate on dextransucrase produced by wild and mutant strains, different concentrations of sucrose were prepared from 73.0 to 730 mM. V_{max} and K_m were determined by plotting the values in Lineweaver Burk plot using Graph Pad Prism (version 5.0). For the determination of optimum temperature, dextransucrase from wild and mutant strains was placed at different temperatures ranging from 15 °C to 40 °C by an increment of 5 °C.

2.9. Determination of molecular weight and in situ electrophoresis

Partially purified dextransucrase was used for the estimation of molecular weight by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) using vertical slab gel system (BioRad, Laboratories Inc., USA). SDS-PAGE was performed according to the method as described by Laemmli (1970) using 6.5% resolving gel. After electrophoresis one half of the gel was stained with Coomassie brilliant blue-R-250 and the other half was used for *in situ* detection of dextransucrase activity band by periodic acid-Schiff staining method as described previously with slight modifications (Holt, Al-Sheikh, & Shin, 2001; Kothari et al., 2011). The gel was washed twice with 0.3 M citrate buffer (pH 5.0) containing 0.5 g/dl triton X-100 and 0.005 g/dl $CaCl_2$ and than incubated in 10.0 g/dl sucrose in the same buffer at 35 °C for 08 h. The gel was washed thrice using 70.0% ethanol with 15 min time intervals. Afterwards it was placed in freshly prepared 0.70% periodic acid and 5.0% acetic acid solution for 01 h at 30 °C and then washed with 0.2% sodium metabisulphite for 05 min. Further the gel was washed with deionized water until yellow background was cleared. The gel was placed in Schiff's reagent (Sigma) in dark for 15 min and washed several times with 0.6% sodium metabisulphite and 3.0% acetic acid until magenta colored bands appeared as activity bands against a clear background. The molecular weight standard used for the estimation of approximate molecular weight was an unstained ruler marker containing 17 recombinant purified proteins (Rec008, Real Biotech Corp., USA). The molecular mass was computed using gel documentation system by BioRad (Model: Universal Hood II) using Quantity One Quantitative software.

3. Results and discussion

3.1. Selection of parent strain

Ten different dextransucrase producing strains were isolated and identified as *L. mesenteroides* on the basis of taxonomic characteristics. All these isolates were screened for dextransucrase production. The dextransucrase activities of theses natural isolates are presented in Fig. 1. One natural variant, *L. mesenteroides* KIBGE IB-22, which was isolated from fermented cabbage showed the highest dextransucrase activity among all other isolates. On the basis of high enzyme activity of extracellular dextransucrase this wild type was selected and subjected for further mutagenesis studies.

All the indigenously isolated strains were characterized on the basis of ribo-typing. A marked variation in terms of nucleotide sequence identity was observed. All the sequences of 16S rDNA of *L. mesenteroides* used in this study were subject to BLAST analysis (http://www.ncbi.nlm.nih.gov/BLAST/) and the results showed that they were all closely related to other reported *L. mesenteroides* specie. Selected sequences were retrieved from NCBI database and multiple sequence alignment and construction of phylogenetic tree showed that the strains are closely related and belong to the family of Firmicutes (Fig. 2).

An initial blast screen of GenBank nucleotide sequence database for wild and mutant stain demonstrate that they both fall in Firmicutes phylum and are closely related to *L. mesenteroides*. On the basis of data collected from NCBI Database and when aligned using Clustal V, KIBGE IB-22 illustrated an 80.8% similarity with *L. mesenteroides* IMAU: 80631 (HM058820) and after mutation KIBGE IB-22M (M20) showed 82.3% similarity with *L. mesenteroides* Moo2 (GU591812) (Table 1).

3.2. UV mutagenesis of wild strain of the L. mesenteroides KIBGE IB-22

Several dilutions of the parent strain cells (KIBGE IB-22) were poured in various petri-plates and were exposed to UV radiation for different time intervals. Similarly, the control for each dilution was also prepared without exposing it to the UV light. The percent survival of the cells from each Petri-plate having different dilutions and exposure time were calculated with reference to control (Table 2). Sufficient numbers of mutants were observed in 10^3 dilution with 2.26% survival rate after 05 s of exposure with UV irradiation. As exposure time increases the rate of percent survival decreased and only 0.1% survival rate was observed in 10^3 dilution after 15 s. However, in the highest dilution factor plate of 10^5, no visible colonies appeared even after 72 h of incubation. Along with this no colony appeared in any other dilution after 30 and 60 sec of exposure time. Colonies that were grown as clusters around the edge of the Petri-plates were not counted. From all the dilutions, 100 colonies that appeared on the Petri-plates were inoculated in the enzyme production medium. All the survivor colonies of mutants were initially tested for dextran and dextransucrase production ability and it was observed that 52 mutants expressed negligible levels of dextransucrase activity. These 52 mutants lost their ability to produce the enzyme upon repeated sub-culturing. The remaining 42 mutants were able to produce different titers of extracellular dextransucrase after several repeated sub-culturing (Fig. 3). Out of these 42 mutants, 27 mutants demonstrated elevated levels of dextransucrase activities whereas, remaining 14 mutants expressed low levels and only 01 (M4) mutant produced

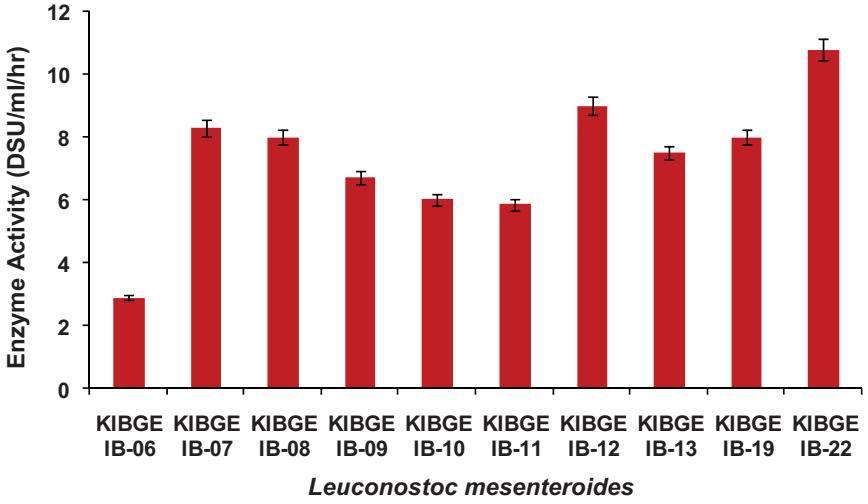

Fig. 1. Production of dextransucrase from various isolates of *L. mesenteroides*.

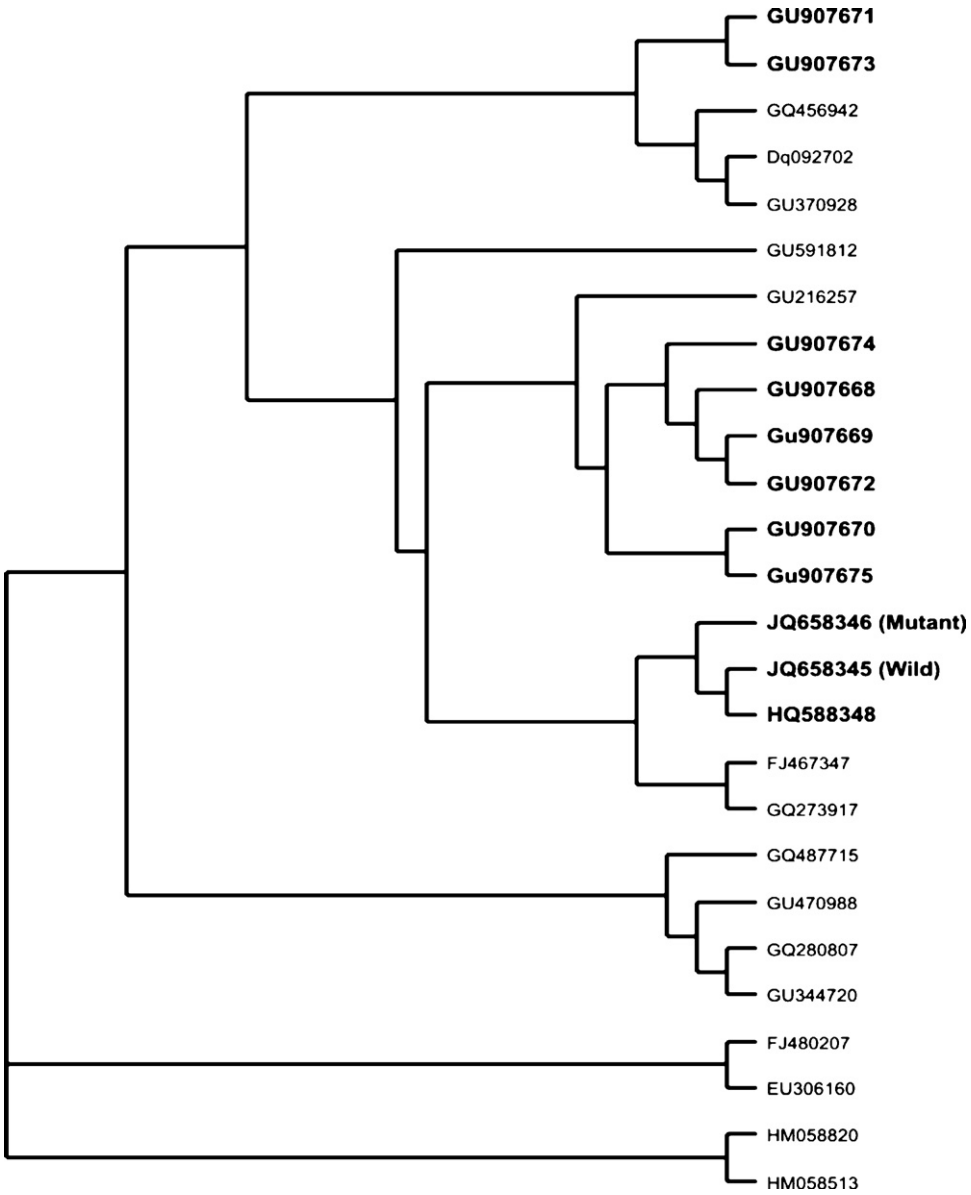

Fig. 2. Phylogenetic tree constructed based on sequencing results of 16S rDNA from indigenously isolated strains of *L. mesenteroides* (bold typed). Sequence of homologous region of reported strains of *L. mesenteroides* were retrieved from NCBI database for comparison.

Table 1
Sequence similarity index of wild and mutant strain of *L. mesenteroides* based on 16S rDNA sequences from available sequence in NCBI GenBank.

Culture name	Base pair	Accession number	Similarity index (%)	
			Wild	Mutant
L. mesenteroides KIBGE-AA1	1482	GU216257	79.8	78.3
L. mesenteroides MGD10-6	1459	HM058513	80.8	79.3
L. mesenteroides Moo2	1370	GU591812	80.3	82.3
L. mesenteroides DM1-2-2	1439	EU306160	80.5	79.3
L. mesenteroides IMAU: 80631	1470	HM058820	80.8	79.2
L. mesenteroides BW2	1459	GQ280807	80.4	79.3
L. mesenteroides 5-3	1015	GQ273917	79.5	75.1
L. mesenteroides WCP907	1520	FJ480207	78.9	79.1
L. mesenteroides KIBGE IB-22	1299	JQ658345	–	77.3
L. mesenteroides KIBGE IB-22M	1438	JQ658346	77.3	–

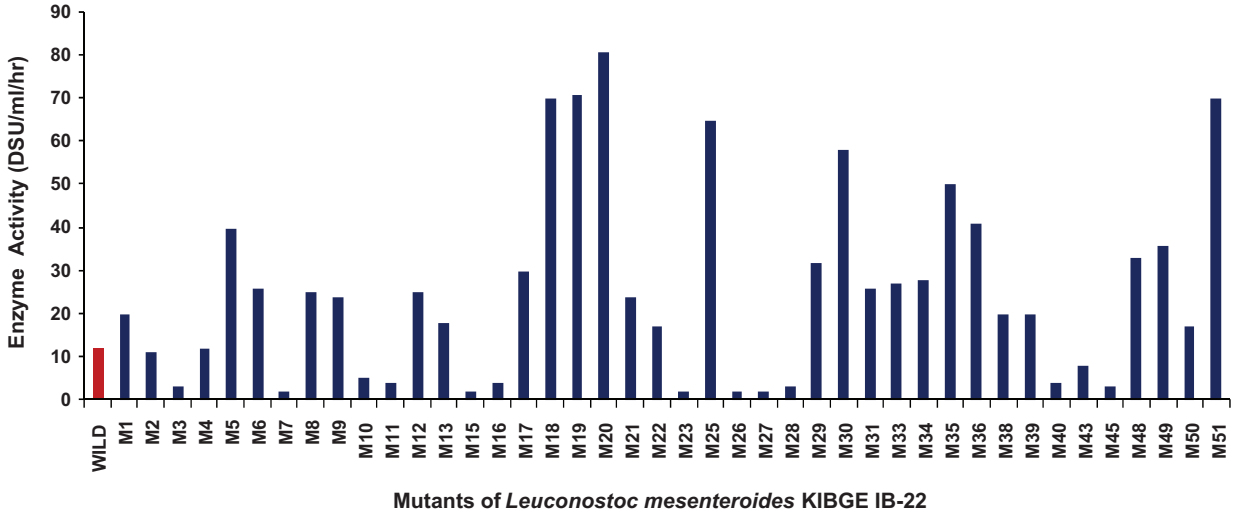

Fig. 3. Dextransucrase activity of wild and mutant strains of *L. mesenteroides*.

the same level of dextransucrase activity as compared to the wild type. Among these 27 strains one prominent mutant strain M20 which was designated as KIBGE IB-22M20, was isolated from the screening plate (dilution: 10^3) that was exposed for 05 s to UV light demonstrated the highest titer of dextransucrase activity. KIBGE IB-22M20 variant significantly exhibited 6.75 times higher extracellular dextransucrase activity (81.0 DSU/ml/h) as compared to the parent strain (12.0 DSU/ml/h) was selected as a hyper-producing

Table 2
Different time intervals of UV light exposure to *Leuconostoc mesenteroides* KIBGE IB-22.

UV light exposure (seconds)	10^3		10^4		10^5	
	Total colonies	Survival%	Total colonies	Survival%	Total colonies	Survival%
Control (wild)	3000	100	1200	100	350	100
5	68	2.26	11	0.91	Nil	Nil
10	13	0.43	05	0.16	Nil	Nil
15	03	0.1	Nil	Nil	Nil	Nil
30	Nil	Nil	Nil	Nil	Nil	Nil
60	Nil	Nil	Nil	Nil	Nil	Nil

Table 3
Partial purification of dextransucrase produced by *Leuconostoc mesenteroides* KIBGE IB-22 (wild) and KIBGE IB-22M20 (mutant).

Purification step	Total enzyme units (DSU)	Total protein (mg)	Specific activity (DSU/mg)	Fold purification	Yield (%)
Wild					
Crude	3710.0 ± 74.2	1354.0 ± 6.77	2.74	1.0	100
PEG-4000	208.0 ± 4.16	6.68 ± 0.033	31.1	11.35	5.60
Mutant					
Crude	25,322.0 ± 506.4	1501.0 ± 7.50	16.87	1.0	100
PEG-4000	6480.0 ± 129.6	37.4 ± 0.187	173.2	10.27	25.6

for further studies. This high expression of dextransucrase by the mutant was observed to be irrevocable as the mutant showed stable enzyme production even after several repeated sub-culturing. The dextransucrase produced by both the wild and the selected mutants (KIBGE IB-22M20) are inducible and require sucrose in the culture broth for enzyme production.

3.3. Partial purification of dextransucrase

Dextransucrase was partially purified to rule out any synergistic effect of the dextran present in the crude preparations of both the wild and mutant strains. Dextransucrase produced by the wild and the mutant strains were concentrated using PEG-4000 which is a non-ionic, water soluble polymer.

The clear culture supernatant (crude) collected after centrifugation contained 3710 and 25,322 dextransucrase units from the wild and mutant strains, respectively. Fold purification and percent yield of the partially purified dextransucrase from the wild and the mutant are summarized in Table 3. Whereas, it was observed that after partial purification the specific activity of dextransucrase from mutant (173.20 DSU/mg) was considerably higher as compared to the wild type (31.10 DSU/mg). However, the percent yield of the enzyme that showed the recovery, did not essentially demonstrated an improved recovery from both the wild and the mutant strains at this step.

3.4. Optimization of kinetic parameters for extracellular dextransucrase activity

In addition to UV mutagenesis, which increased the dextransucrase activity, the data presented in this communication also focuses to note any change occurred in the kinetic behavior of the enzyme produced by mutant with reference to wild type. For this

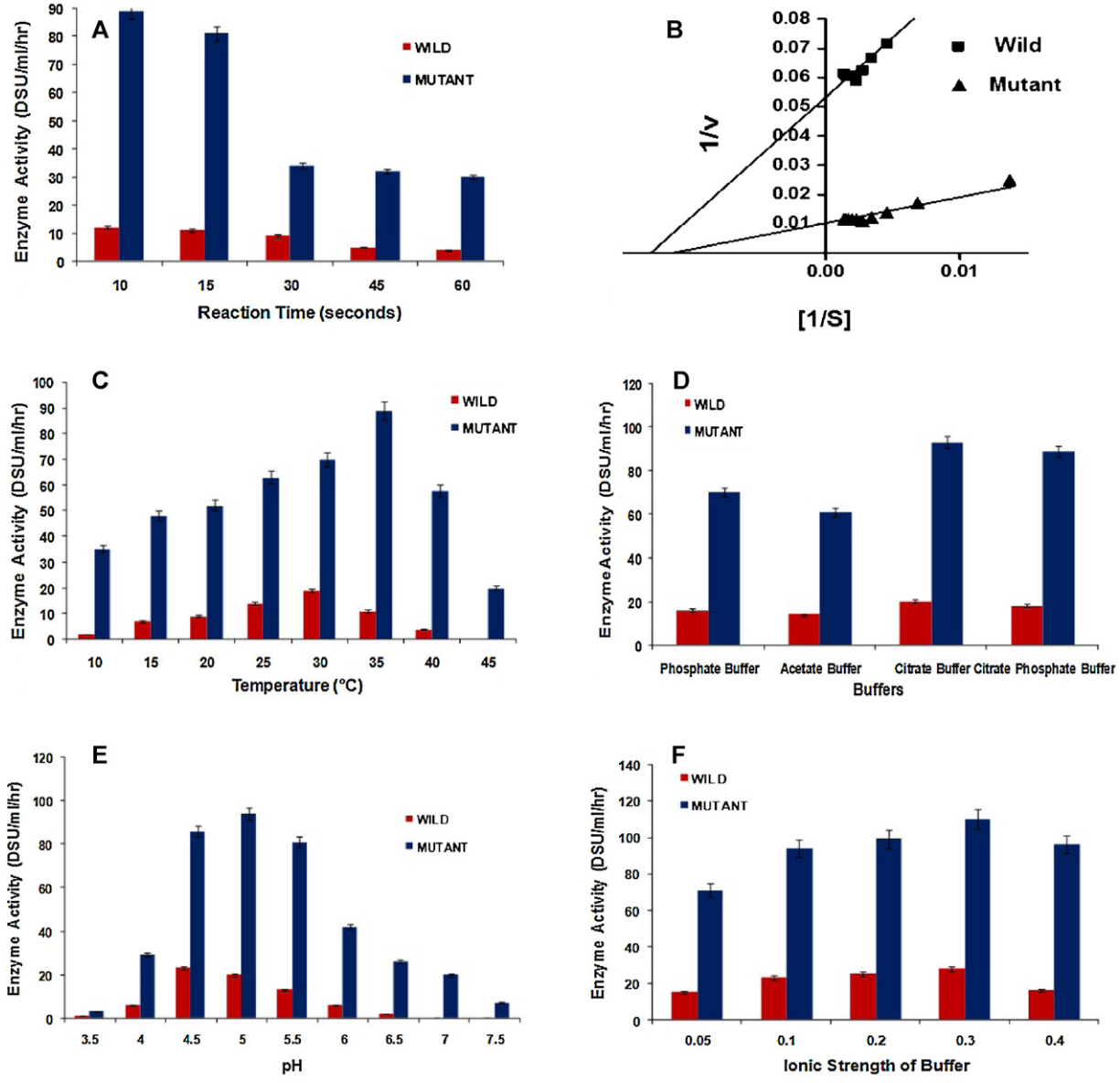

Fig. 4. Comparison of kinetic constants of dextransucrase produced by wild and mutant strains of *L. mesenteroides*. (A) Dextransucrase activity of wild and mutant strains of *L. mesenteroides* at different time intervals; (B) Lineweaver Burk Plot of dextransucrase from *L. mesenteroides* KIBGE IB-22 (wild) and KIBGE IB-22M (mutant); (C) dextransucrase activity of wild and mutant strains of *L. mesenteroides* at different temperatures; (D) dextransucrase activity of wild and mutant strains of *L. mesenteroides* with different buffers; (E) dextransucrase activity of wild and mutant strains of *L. mesenteroides* at different pH values; (F) dextransucrase activity of wild and mutant strains of *L. mesenteroides* at different ionic strength values of the buffer.

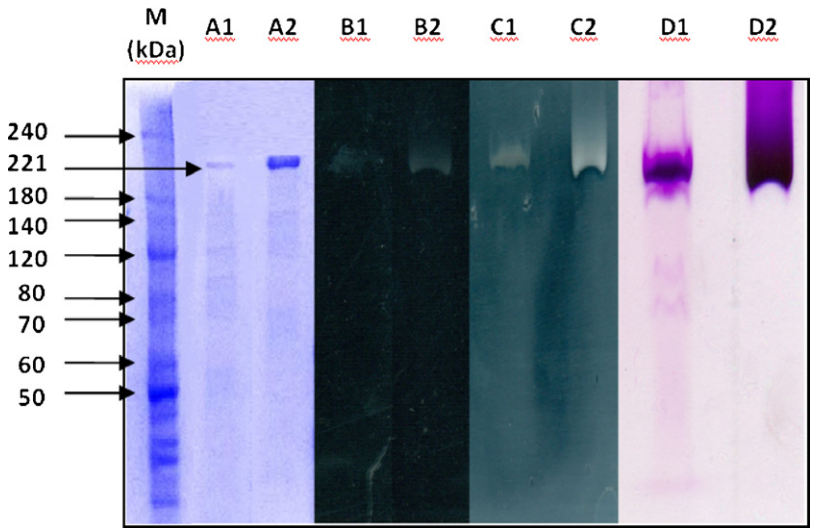

Fig. 5. SDS-PAGE profile of dextransucrase produced by *L. mesenteroides* KIBGE IB-22 (wild) and KIBGE IB-22M20 (mutant). Lane M, molecular weight standards; Lanes A1 (wild) and A2 (mutant) Coomassie blue staining; Lanes B1 (wild) and B2 (mutant) gel placed in 10.0% sucrose; Lanes C1 (wild) and C2 (mutant) gel placed in 70.0% methanol showing dextran synthesis; Lanes D1 (wild) and D2 (mutant) Schiff's staining. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

purpose different kinetic parameters were studied to determine any change in the catalytic property of the enzyme.

Reaction time for dextransucrase was determined and it was observed that both wild and mutant showed maximum enzyme activity at 10 min (Fig. 4A). It is very significant to determine the exact time required for dextransucrase and sucrose to react under specific conditions for the production of dextran (product) because in the reaction mixture sucrose is broken down into glucose and fructose. The glucose molecules link together to form dextran polymer in a specific time period and fructose in the reaction mixture is accumulated. After this specific time period when glucose is in limited concentration, accumulated fructose starts acting as an inhibitor and inhibits further linkage of free glucose to dextran chain and competes against glucose for biopolymer extension.

Different concentrations of sucrose were used to determine the substrate saturation kinetic of the dextransucrase produced by both the wild and mutant type. The apparent V_{max} and K_m values were computed by using Lineweaver Burk double reciprocal plot for both, wild and mutant. The effect of substrate concentration on the velocity of the reaction and Lineweaver Burk plot for wild type and mutant dextransucrase is presented in Fig. 4B and it was observed that both wild and mutant demonstrate extremely different values. Using purified sucrose the apparent V_{max} and K_m values for dextransucrase from wild were 18.84 DSU/ml/h and 77.09 mM with standard error of 0.39 DSU/ml/h and 8.05 mM, respectively with R^2 0.99. Whereas, V_{max} and K_m values for the mutant were calculated as 104.2 DSU/ml/h and 101.7 mM, respectively and these values were very high as compared to the saturation kinetics of dextransucrase from wild type. Standard error for V_{max} and K_m for dextransucrase from mutant was 4.11 DSU/ml/h and 17.2 mM, respectively with R^2 value of 0.98. A wide range of substrate concentration has been previously reported for extracellular dextransucrase. The K_m value for two previously reported dextransucrase enzymes designated as I and II were 10.7 and 250 mM, respectively (Kobayashi & Matsuda, 1975). For dextransucrase from *Streptococcus mutans* FA1 and *Streptococcus sanguis* the K_m was 55 mM and 4.8 mM, respectively (Carlsson, Newbrun, & Krasse, 1969; Scales, Long, & Edwards, 1975). Dextransucrase produced by other *L. mesenteroides* strains has also demonstrated a wide range for the initial rate of reaction such as *L. mesenteroides* NRRL 512 have K_m of 12–16 mM and *L. mesenteroides* NRRL B-1299 have 13.1–13.9 mM (Kobayashi & Matsuda, 1976; Miller, Eklund, & Robyt, 1986).

Dextransucrase activity of wild and mutant strains was measured at a broad temperature range and it was observed that the optimum temperature for both wild and mutant dextransucrase was different. Dextransucrase from wild showed maximum activity at 30 °C while, mutant at 35 °C and after reaching maximum the activity declined (Fig. 4C). After optimum temperature the decrease in the enzyme activity is due to the instability of the dextransucrase because elevated temperature usually generates enough disturbances to break the intra-molecular attractions between polar and non polar groups within the protein structure. Due to this instability some conformational alterations occur at the active site of the enzyme and hence render enzyme substrate interaction. This slight change in optimal temperature for both, wild (30 °C) and mutant (35 °C) may be due to the mutation occurred at the gene level. The temperature optima of other reported dextransucrases from various strains is generally in between 30 and 40 °C (Itaya & Yamamoto, 1975; Kim & Robyt, 1995; Kobayashi & Matsuda, 1976).

Four different buffers were examined for the maximum dextransucrase activity by KIBGE IB-22 and KIBGE IB-22M20 (Fig. 4D). Dextransucrase from wild type showed slight differences in the activities with different buffers and exhibited higher enzyme activity in citrate buffer and mutant also demonstrated the highest enzyme activity in the same buffer. The optimum pH for the reaction of dextransucrase, produced by both wild and mutant strains, was somewhat different. KIBGE IB-22 (wild) showed an optimum pH value at 4.5 whereas; mutant KIBGE IB-22M20 exhibited at pH value of 5.0. One most interesting feature was also noticed that the KIBGE IB-22M20 not only exhibited dextransucrase activity toward the acidic side but also showed 21.3% (20.0 DSU/ml/h) and 7.5% (7.0 DSU/ml/h) activity toward neutral pH values at 7.0 and 7.5, respectively. However, no enzyme activity was detected at neutral pH by the wild strain (Fig. 4E). The optimal ionic strength for both the wild and the mutant strains was similar. It was observed that the enzyme produced by them can work even at higher ionic strength of 0.3 M (Fig. 4F).

3.5. Molecular weight

SDS-PAGE was performed for the molecular weight of partially purified dextransucrase from wild and mutant. It was found that enzyme from both the wild and mutant strain, KIBGE IB-22 (parent) and KIBGE IB-22M20 (mutant), had same molecular weight of 221 kDa and mutation did not changed the molecular weight, although the intensity of the bands increased in case of mutant (Fig. 5, Lanes A1 and A2). After incubating the gel in sucrose it was observed that a very faint white band was visible for the wild as compared to the prominent activity band of the mutant strain (Fig. 5, Lanes B1 and B2). Further confirmation was achieved by introducing the gel in 70.0% ethanol that actually precipitated the dextran in the gel and clear white bands were observed (Fig. 5, Lanes C1 and C2). In periodic acid-Schiff's staining the wild strain showed two faint glycoprotein bands of approximately 70–90 kDa whereas, they were not produced by mutant strain. Mutant strain showed only one single magenta band corresponding to 221 kDa band of dextransucrase with no other glycoprotein (Fig. 5, Lanes D1 and D2).

4. Conclusions

Current investigation demonstrates successful use of UV irradiation for the generation of effective mutants that were capable of producing increased yields of dextransucrase. The comparison of kinetic parameters of dextransucrase activity of wild and mutant strains is also reported. The mutants generated showed their enhanced enzyme production capability. Mutants generated (100) after exposure to UV irradiation of the parent strain were screened and tested for any modification in the reputational nature of dextransucrase synthesis. About 42 mutants showed dextransucrase activity and among them 15 mutants exhibited lower and 27 mutants demonstrate enhanced enzyme activity as compared with parent strain (wild). *L. mesenteroides* KIBGE IB-22M20 was the most promising mutant that illustrated elevated level of dextransucrase activity among all the generated mutants. Current investigation showed that this exceptional mutant has a prominent future for the production of high titers of dextransucrase. Dextran is a novel biopolymer with potential commercial interest and in subsequent study the dextran produced from both wild and mutant strains will be characterized in order to expedite feasibility for the commercial production of dextran by this newly discovered mutant. Further studies on characterization of dextran produced by this mutant will provide useful information regarding the changes occurred due to mutagenesis.

Acknowledgement

Authors are grateful to Dr. Abdul Hameed, Principal Scientist, IB&GE, Islamabad for providing sequencing facilities.

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