Role of nutrients and environmental conditions for the production of dextransucrase from *L. mesenteroides* KIBGE-IB26

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Abstract: The bacterial strains capable of producing dextransucrase enzyme were isolated from different fruits and vegetables sources. In primary screening, five strains were selected on the basis dextransucrase production and among them *L. mesenteroides* KIBGE- IB26 isolated from bottle gourd (Lagenaria Vulgaris) was selected for further studies. For the enhancement of enzyme production, different physicochemical parameters were optimized. Maximum production of dextransucrase was achieved after 06 hrs using sucrose (20.0g/l) as a substrate at 25°C. Maximum dextransucrase production was achieved when medium pH was kept 7.5 before sterilization. In addition, medium was also supplemented with $CaCl_2$ and K_2HPO_4 and maximum enzyme production was achieved at 0.0025g/dl calcium chloride and 2.0g/dl K_2HPO_4 with enzyme activity of 87 DSU/ml/hr. Production of dextransucrase in shorter period of time makes this strain an attractive candidate for commercial production of dextransucrase.

Keywords: Dextransucrase, Dextran, *L. mesenteroides, Glucansucrase.*

INTRODUCTION

Glucansucrases [2.4.1.5] are large extra cellular enzymes from glycoside-hydrolase family 70 that are capable of producing various glucans like dextran, mutan and alternan etc. from sucrose (Monsan, 2001). This family has diversity in nature, it has enzymes that are able to synthesize glycosidic bonds like α-1, 2, α-1, 3, α-1, 4 and α-1, 6 linkages. Therefore, according to the enzyme specificity different kind of dextran can be produced that are different in term of size, structure and degree of branching (Claire, 2006).

Dextran is the polymer of D-glucopyranose units that are linked predominantly by α-1, 6 glycosidic linkage in the main chain. Both dextransucrases and dextran have various applications in different industries like food, pharmaceutical, cosmetics and agriculture etc. Application of dextran in various industries is molecular weight dependent as in pharmaceutical industry dextrans of molecular weight 40 and 70 kDa are used as blood flow improver and blood plasma extender, respectively. Dextran of molecular weight 70±25kDa classified as clinical grade dextran (Robyt, 1986).

Dextransucrase has been reported from many species of different genera like *Lactobacillus, Leuconostoc* and *Streptococcus.* Among these species it is constitutive in streptococcus while inducible in *Leuconostoc* species and produce only in the presence of sucrose in the fermentation medium (Ciardi, 1997; Chellapandian, 1998; Kim & Robyt, 1994). Some strains of *Leuconostoc mesenteroides* produce only one type of dextransucrase and dextran while other can produce different type of dextransucrase and dextrans (glucan). Differences in the types are manifest by heterogeneity in dextran solubility profiles and electrophoretic band patterns of glucansucrase (Kato, 1990).

Leuconostoc mesenteroides strains can grow between 5-30°C but the optimum temperature for growth is between 25-30°C. Dextransucrase from *Leuconostoc mesenteroides* NRRL B-512 (F) is most commonly used for industrial production of dextran. This strain produces enzyme in large quantity with minimum number and amount of other proteins and it synthesized a highly liner high molecular weight soluble dextran. Dextran synthesized from this strain has 95% α-1, 6 glycosidic linkage. This may be distinguished from other strain that produce more than one type of enzyme in different ratio like levansucrase and invertase and produce both soluble and insoluble dextran (Sidebotham, 1974; Jeanes, 1954; Guggenheim, 1967).

Present study is design to keeping its clinical and commercial application in mind, to enhance the production of dextransucrase from *L. mesenteroides* KIBGE-IB26 strain isolated from indigenous source.

MATERIALS AND METHODS

Selection, identification and taxonomic characterization of strain

Dextran producing epiphytic bacterial strains were isolated from various fruits and vegetables and inoculated

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in 10% sucrose broth and incubated at 25°C. Pure culture of isolates were obtained after serial dilution and standard spread plate technique on 1.0% sucrose agar plates supplemented with sodium azide (0.001%) (Qader *et al*, 2001). Colonies that appeared as slimy, shiny and pinpointed were selected for enzyme production by inoculating in 2.0% and 10.0% sucrose broth. For further confirmation by 16S rDNA sequence analysis was also performed and sequence was submitted to Gen Bank with accession number of KF241862. Phylogenic tree was constructed using NCBI database of previously reported strains of *L. mesenteroides*. Complete taxonomic studies and identification of the isolates were carried out according to "Bergey's manual of determinative bacteriology" (Holt, 1994). Similarity index analysis of 16S rDNA of selected strains was also done by using nucleotide BLAST facility from National Center for Biotechnology Information (NCBI).

Optimization of medium for dextransucrase production

Leuconostoc mesenteroides KIBGE-IB 26 grown in basal liquid medium containing (g/l): sucrose 25.0, Bacto-peptone 5.0; Yeast extract 5.0, K_2HPO_4 15.0, $CaCl_2$ 0.1, $MgSO_4$ 0.01, $MnCl_2$0.01, NaCl0.01 and pH was 7.5 before sterilization (Aman *et al*, 2009). This medium was further optimized for maximum production of enzyme by varying the concentration of one component at a time methodology. Physical parameters like temperature, pH, and Incubation time were also varying in stepwise manner to optimize the growth conditions for enzyme production (Table 1). The modified medium contain following composition (g/l) sucrose 20.0, peptone 5.0, yeast extract 5.0, K_2HPO_4 20.0, $CaCl_2$ 0.175, $MgSO_4$ 0.01, $MnCl_2$ 0.01, NaCl 0.01 and pH of the medium adjusted to 7.5 before sterilization.

Experimental design for enzyme production

For the production of dextransucrase, 100 ml modified medium containing sucrose (2.0%) was inoculated with pure culture and incubated at 25°C for 24 hours. This inoculum was then transferred into 900ml fresh 2.0% sucrose medium and incubated for 18 hours at 25°C. Fermented broth was centrifuged at 15,000×g and dextransucrase activity was determined in cell free filtrate (CFF).

Enzyme activity

Enzyme activity was performed by mixing 0.05ml CFF to 1.0 ml substrate (125mg/ml sucrose in 150mM succinate buffer, pH 5.0) and incubated at 35°C for 10 minutes. Reaction was stopped by adding 1.0N NaOH and reducing sugar was estimated according to the method described earlier (Kobayashi & Matsuda, 1974).

Units of the enzyme activity are expressed in DSU. "One unit of enzyme is equal to the amount of enzyme that is required for converting 1.0 milligram of sucrose into fructose and dextran in 1.0 hr at 35°C under standard assay conditions (Lopez & Monsan, 1980).

Protein estimation

Total protein of cell free filtrate (CFF) was estimated by Lowry's method and bovine serum albumin was used as standard (Lowry, 1951).

RESULTS

Selection of strain

The bacterial strain *Leuconostoc mesenteroides* KIBGE-IB26 used in current study was isolated from bottle gourd (Lagenaria Vulgaris). The strain was selected on the basis of high yield of dextransucrase (fig. 1). Microscopic studies showed that strain was a gram-positive cooci arranged in chains or diplococci. In 1% sucrose agar, isolate was appeared as slimy shiny and smooth, pinpointed colonies while 10% sucrose medium became viscous at the end of fermentation. Biochemical tests revealed that isolated strain did not hydrolyse the H_2O_2 and vancomycin resistant, although all the tested saccharides were metabolised (table 2). All these characters showed that isolate was belong to *Leuconostoc mesenteroides* specie. 16S rDNA analysis sequence also showed 98% similarity with previously deposited sequences of *Leuconostoc mesenteroides,* which confirmed the identification of strain. Phylogenic tree was also constructed with other isolates of *L. mesenteroides* strain (fig. 2).

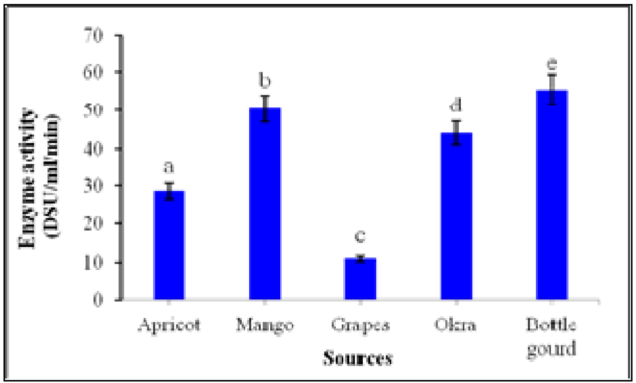

Fig. 1: Production of enzyme using different sources from *Leuconostoc mesenteroides* KIBGE-IB26.

Effect of temperature and pH on dextransucrase production

Temperature plays an important role in cell growth and survival rate. To identify the optimum temperature for dextransucrase production, fermentation was carried out at different temperatures and maximum enzyme production was achieved at 25°C. Sharp declined in enzyme production was observed with increase in temperature and production was retarded at 35°C (fig. 3A). These results indicated that the strain is temperature sensitive and can grow in narrow range of temperature or dextransucrase produced is thermo labile.

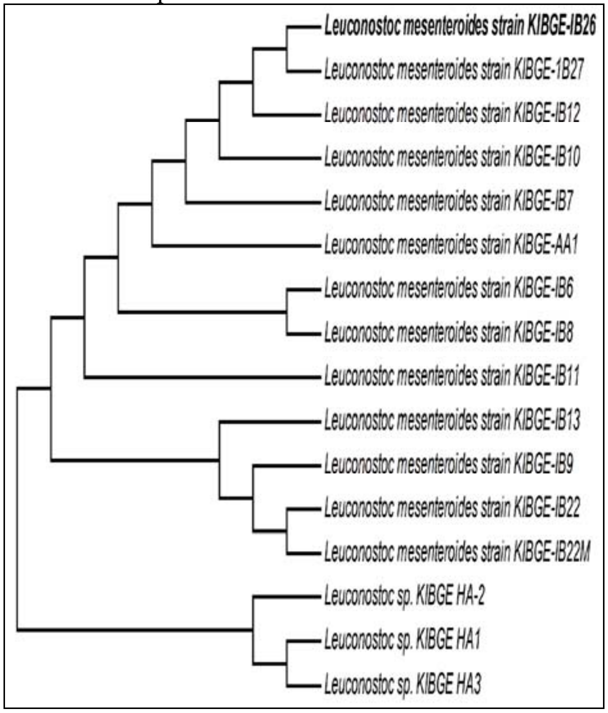

Fig. 2: Phylogenic tree constructed on the basis of 16S rDNA

To observe the effect of initial medium pH on dextransucrase production, culture was inoculated in sucrose broth having different pH. Maximum yield of enzyme was obtained at slightly neutral pH (7.5) while at highly acidic (pH 5.0) and alkaline pH (9.0) enzyme production was very low (fig. 3B).

Effect of incubation time on dextransucrase production

Time course play important role in the production of dextransucrase and biomass. The result showed that the biomass and enzyme production is directly proportional to each other when biomass reached to maximum, enzyme yield was also improved. Biomass increased rapidly and reached to maximum after 6.0 hours of fermentation with a wet cell mass of 0.86g/dl (fig. 4). According to bacterial growth curved during exponential phase bacterial cells increased in numbers rapidly and after stationary phase become decline. As a result, the production of enzyme was also increased during exponential phase and in the early stationary phase of cell growth. Thus, the maximum dextransucrase production (59.0 DSU/ml/hr) was observed after 6.0 hours.

Effect of sucrose concentration on dextransucrase production

Sucrose concentration was varied in fermentation medium and maximum production of dextransucrase was observed at 2.0 % substrate concentration (fig. 5).

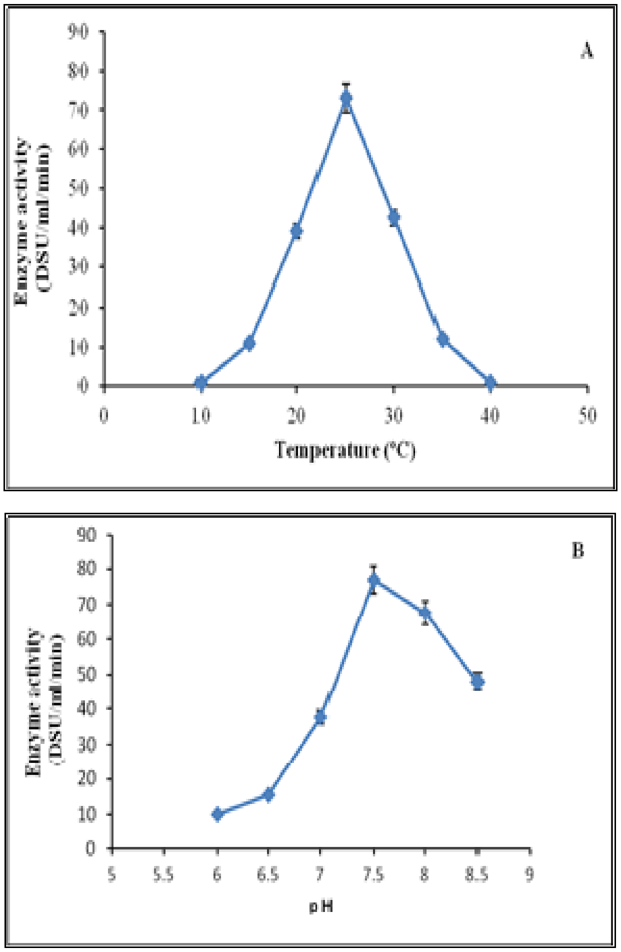

Fig. 3: Effect of temperature (A) and pH (B) on production of dextransucrase from *Leuconostoc mesenteroides* KIBGE-IB26

Effect of nitrogen sources on dextransucrase production

Nitrogen sources play key role in cell multiplication and metabolism. Presence of nitrogen and phosphorus in appropriate ratio enhance the bacterial growth. In fermentation broth, when yeast and peptone were added in combination, production of dextransucrase increased markedly. Both contain different types of vitamins and minerals that support the bacterial growth. But further increased in yeast and peptone concentration in medium, declined the enzyme production (figs. 6A and 6B).

Effect of K_2HPO_4 on enzyme production

When K_2HPO_4 was added in fermentation medium dextransucrase production was increased gradually and at 2.0% maximum production was achieved while as the 3.0% K_2HPO_4 was added in the fermentation medium, decreased in enzyme production (25%) was observed (fig. 7A). This decline in enzyme production could be due to the excess of potassium ions that produce more alkaline environment in the medium resulting in decreased in cell growth.

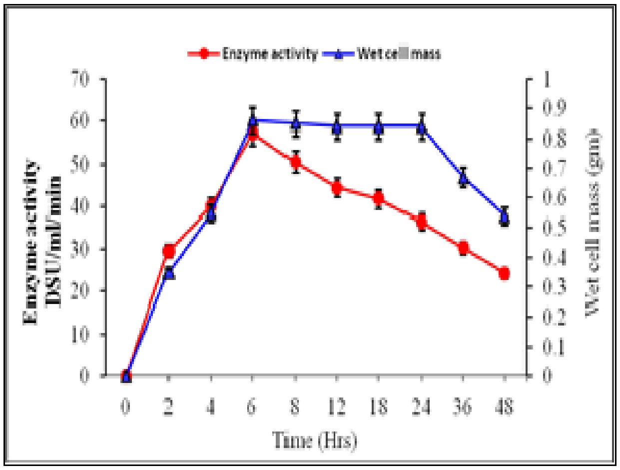

Fig. 4: Effect of incubation time on dextransucrase and cell mass production from *Leuconostoc mesenteroides* KIBGE-IB26

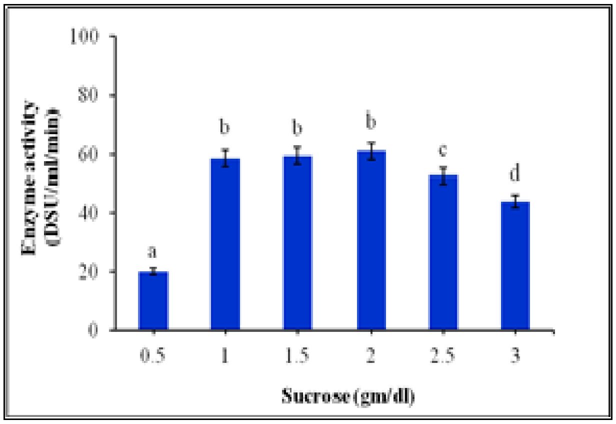

Fig. 5: Effect of sucrose concentration on dextransucrase production from *Leuconostoc mesenteroides* KIBGE-IB26

Effect of $CaCl_2$ on enzyme production

Calcium ions play important role in enzyme stability and it was also reported that calcium ions have been used for thermal stability of enzymes (Kawamoto, 2001). Calcium ion was incorporated in the fermentation medium as calcium chloride in different concentration ranging from 0.0025% to 0.0175%. It was found that maximum enzyme production was achieved at 0.0025gm/dl calcium chloride with enzyme activity of 87DSU/ml/hr (fig. 7B). However, further addition of calcium chloride (0.0125g/dl) in fermentation medium created a negative impact on the production and less dextransucrase production (27%) was observed.

DISCUSSION

Physiochemical parameters of *L. mesenteroides* KIBGE-IB 26 strain, isolated from bottle gourd were studied and it was observed that it can grow between 15-30°C while the optimum temperature is 25°C, above this temperature the growth of bacteria retarded because *L. mesenteroides* belong to lactic acid bacteria that have low guanine-cytosine content so cannot survive on elevated temperatures and strain can produce dextransucrase at narrow range of pH and temperature.

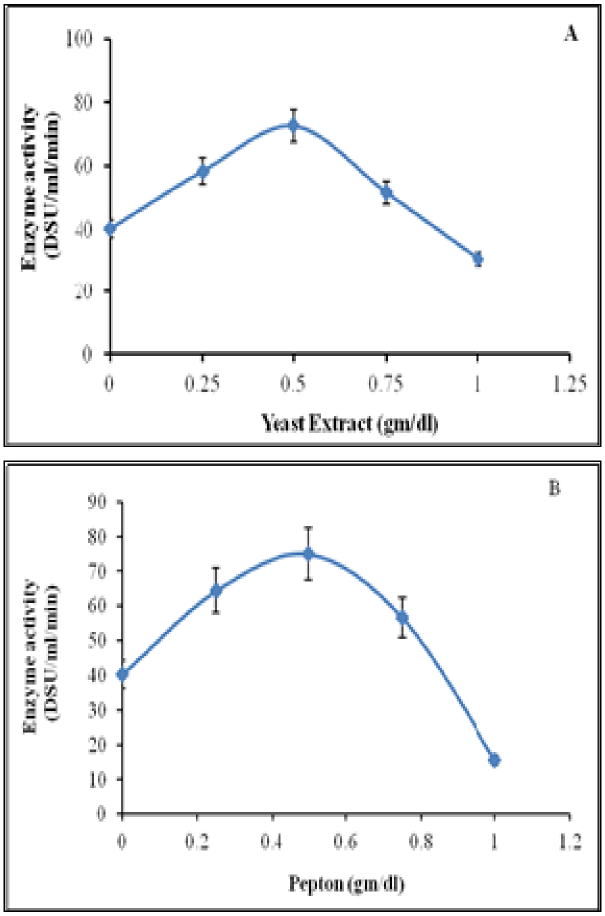

Fig. 6: Effect of yeast extract (A) and peptone (B) on dextransucrase production from *Leuconostoc mesenteroides* KIBGE-IB26.

It may be due to the fact that the optimum pH for the growth of *L. mesenteroides* is 6.0 to 6.9 and initial pH of medium >7.5 is suboptimal since at pH greater than 7.5 lag phase of the bacteria exponentially increased (Wolf and Fagler, 2001; Tsuchiya *et al*, 1952). Consequently, at initial medium pH below 7.0 decreased in enzyme production might be due to the morphological and physiological changes in bacterial cell and only few organisms that adapted the environmental changes can survive. Thus, the yield of enzyme at extreme pH values was very low (Salem, *et al* 1984). *L. mesenteroides* KIBGE-IB26 produced maximum dextransucrase in 6 hours and it is a distinguish quality of this strain with reference to previously reported strains of *L. mesenteroides* like PCSIR 3, NRRL B-512(F) and AA1 that produced maximum dextransucrase in 18,12 and 8 hours, respectively at 25°C (Qader *et al*. 2001, Mariana. 2000).

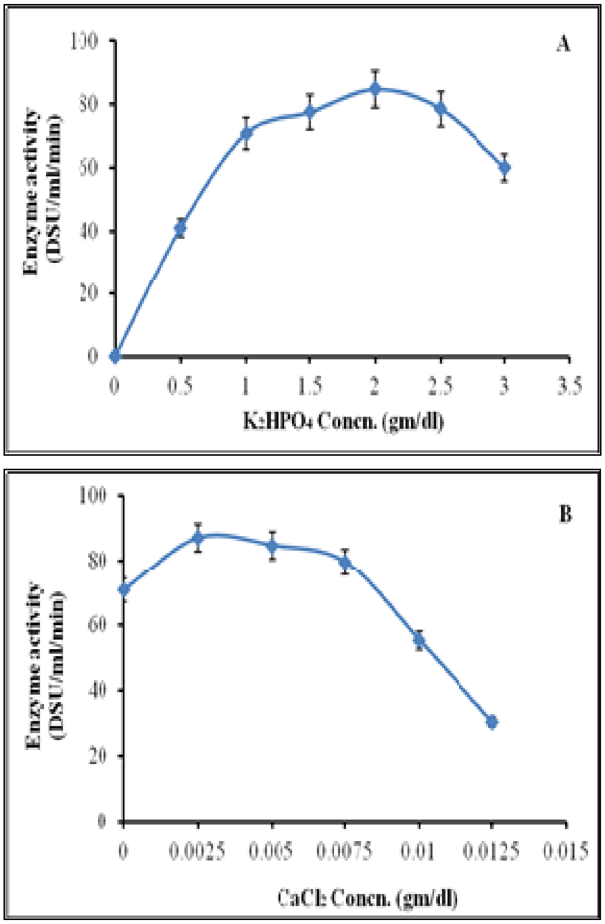

Fig. 7: Effect of K_2HPO_4 (A) and $CaCl_2$ (B) on dextransucrase production from *Leuconostoc mesenteroides* KIBGE-IB26.

It has been reported earlier that sucrose is the only substrate for the induction of extra cellular dextransucrase from *L. mesenteroides.* Furthermore, many other strains have been reported that produced maximum dextransucrase at 2.0% sucrose concentration (Kim, & Robyt, 1994; Qader *et al*. 2001; Lopretti *et al.*, 1999 & Michelena *et al.*, 2003). It was also reported that the bacterial cell uses 85% of available sucrose for the production of dextransucrase during fermentation (Lopez & Monsan, 1980; Neely & Nott, 1962). Therefore, further increase in substrate from optimum concentration not only accumulated in the medium but also can cause substrate inhibitory effect. This might be due to the lowering of available water (a_w) in fermentation medium which resulted an increase in lag phase which causes decrease in specific growth rate in batch fermentation (Wolf and Fogler, 2001). During fermentation, dextran Production in medium is also a major problem and at high substrate concentration the high dextran production occurred which ultimately increases the viscosity of the medium, which hindered the transportation of nutrient into bacterial cell and cannot satisfy metabolic demand of the cell. As a consequence, the multiplication of cell and enzyme production is retarded at above the optimum concentration of substrate. Lopretti (1999) and Michelena (2003) have reported that due to the viscosity of fermentation medium both the growth and enzyme production is declined. Incorporation of both yeast extract and peptone, which contain vitamins and mineral into medium supported the growth of *L. mesenteroides* KIBGE- IB26 and increased the production of enzyme. It was found that the incorporation of only one nitrogen source is also insufficient for maximum yield of dextransucrase as reported by Qader *et al* (2006).

There is critical pH (6.7-6.9) that favors the growth of the *L. mesenteroides* species as at this pH exponential growth began but during fermentation different organic acid were produced that decreased the pH of the fermentation medium up to pH 4.2 or 4.3, which ultimately decreases the dextransucrase production (Tsuchiya, 1952; Veljkovic, 1992). The addition of K_2HPO_4 in medium produced a buffering effect and kept the pH value above 5.0 that favors the cells growth and prevent enzyme denaturing (Rodrigues *et al.*, 2003). On the other hand, It was observed that the enzyme production was also increased after the addition of $CaCl_2$ into the medium because Ca^{+2} ions stabilizes the three dimensional structure of enzyme hence, after the addition of Ca^{+2} ions in medium the recovery of the enzyme increased. Seo *et al.* (2009) has also reported that calcium ions have neutralizing effect in acidic solution therefore, neutralize the lactic acid that produced during fermentation by lactic acid bacteria. Dols *et al.* and Lopez *et al.* (1998, 1999) used 0.005% $CaCl_2$ in fermentation medium for the optimum production of enzyme from *L. mesenteroides*.

CONCLUSIONS

Current study was design to isolate a bacterial strain that is capable of producing extra cellular dextransucrase enzyme. After optimization of physicochemical parameters of fermentation the maximum production of enzyme from KBGE IB-26 was recorded in 06 hours at 25°C when initial medium pH was kept 7.5 before sterilization. Therefore it could be the ideal candidate for bulk production of dextransucrase enzyme.

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