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Production of Dextransucrase by Locally Isolated Bacterial Strain

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ABSTRACT

Dextransucrase is used for the production of dextran, a commercially important product, with numerous applications in many industries such as food, medical, pharmaceutical, and cosmetics. In the current study, dextransucrase producing microorganisms were separated from different vegetables, fruits, and milk samples collected from the local market of Lahore, Pakistan. Sucrose-based medium, supplemented with vancomycin and tetracycline, was used for the selective growth of *Leuconostoc* species. The obtained isolates were screened by using the shake flask method. The isolate IIB-C9 was selected for the parametric optimization as it gave the maximum activity. Among the 10 different fermentation media screened for enzyme production, maximum dextransucrase activity (9.96 U/ml) was obtained by bacterial strain IIB-C9 in fermentation medium M6 comprising (yeast extract 15 g/L, sucrose 40 g/L, and Na_2HPO_4 20 g/L). The optimized temperature and initial pH were 30°C and 6.95, sequentially. The IIB-C9 isolate gave optimum production of dextransucrase when M6 medium was accompanied with 2% yeast extract and 5% sucrose followed by 24 hours of incubation at 160 rpm in a shaking incubator. The bacterial isolate IIB-C9 was identified as *Leuconostoc* species based on biochemical characters.

Keywords: *Leuconostoc* species, Isolation, Screening, Submerged fermentation, Dextransucrase, Fructose Standard Curve, Biochemical characteristics, Optimization

INTRODUCTION

Dextransucrase (EC.2.4.1.5), which belongs to the glucansucrase family, is an extracellular enzyme that uses sucrose as a substrate and converts it into fructose and dextran. The enzymatic degradation of sucrose into dextran and fructose is shown in Equation 1 below (Santos *et al*., 2000).

$$\underset{\text{Sucrose}}{n\,C_{12}H_{12}O_{11}} \xrightarrow{\text{Enzyme}} \underset{\text{Dextran}}{(C_6H_{10}O_5)_n} + \underset{\text{Fructose}}{n\,C_6H_{12}O_6}$$

Different microbial species can produce extracellular or intracellular dextransucrase. But the extracellular production of dextransucrase shows more activity in comparison to the intracellular production. The average molecular mass of the enzyme is 170 kDa. Mostly the enzymes present in this family, utilize sucrose as the D-glucopyranosyl contributor to produce D-glucans of high molecular mass and also produce reducing sugar (fructose). This results in the production of glucan polysaccharides by using the glucose entity of sucrose. Hence, also known as glucosyltransferases (GFT) (Leemhuis *et al*., 2013; Cantarel *et al*., 2009). According to CAZy (carbohydrate-active enzymes) classification scheme, glucansucrases are classified as the glycosidic hydrolase family 70 (GH70) enzymes, formed on similarity in amino acid sequences (Stam *et al*., 2006; Vujicic-Zagar *et al*., 2010).

Several bacterial species are reported for the fabrication of dextransucrase especially those belonging to the genus *Leuconostoc* and *Lactobacilli,* are noticeable (Goyal *et al*., 1995; Purama and Goyal, 2009). Two important species of *Streptococcus* i.e. *S. mutans* (Kang *et al*., 2006) and *Streptococcus* OMZ 51 (Sidebotham and Weigel, 1974) are reported for the production of dextransucrase. Furthermore, *Lactobacillus casei* (Hammond, 1969), *Enterococcus faecalis* (Hashem *et al.*, 2016) *Weissella cibria* (Kang *et al*., 2006), *W. kinchii* F28, and *W. confusa* are also reported for the production of this enzyme (Hongpattarakere *et al*., 2012). Whereas, *L.mesenteroides* and *L.dextanicum* are commonly used for the production of dextransucrase in the genus *Leuconostoc* (Goyal *et al*., 1995; Purama and Goyal, 2008). *L. mesenteroides* dextransucrase is of industrial importance (Giavasis, 2013). This strain shows the highest activity of the dextransucrase.

Author Contribution: **A.M.,** Data collection and analysis and manuscript writing; **Z.K.,** Data collection, manuscript writing and finalizing; **N.M.,** Concept generation, study designing, data analysis manuscript writing and finalizing.

Dextransucrase producing bacteria can be isolated from vegetables (cucumber, cabbage, eggplant, and pepper) grapes, milk, sugarcane, yogurt and milk. Cashew, apple and whey are also reported for the production of dextransucrase. New strains are being explored for the best production of this enzyme.

Dextransucrase is the only industrial enzyme that utilized sucrose as a substrate for the commercial synthesis of dextran (Robyt *et al*., 2008). Dextran has a complex structure. The term dextran is collectively used to describe a large class of hydrocolloid homopolysaccharides that are extracellularly produced by bacteria. Many glucose molecules build this branched structure. A linear backbone consists of at least 50% α-1,6 glucosidic bonds and α-1,2, α-1,3 or α-1,4 linked branches are present in the straight chain of dextran (Bounaix *et al*., 2010; Vettori *et al*., 2012). Dextran has numerous applications. Dextran can be utilized as a sweetener, food preservative, and cryo-protectant in the food industry. Mixing 2 to 4% dextran with ice-creams has many valuable properties of maintaining their viscosity (Ramalingam *et al*., 2018). By utilizing the dextran conjugating proteins, the physical and chemical properties of protein like heat stability, emulsification, antioxidant and functional properties are improved (Kothari et al., 2014). Dextran can be used as a stabilizing, bulking agent, and immune-stimulating agent in cosmetic and pharmaceutical industries. The commixtures of dextran sulfate and escin are used to treat skin diseases, skin radicals, rough and flaky skin, edema of the skin, and dark rings around the eyes (Renault & Mauric, 2013). Dextran causes a rapid increase in plasma volume by lowering the hematocrit and blood viscosity (Evarts, 1967). It also improves the blood flow in the body (Boschenstein *et al*., 1966). Dextran can be used to make prebiotic compounds, artificial blood (Bhavani & Nisha, 2010), Sephadex gel (Shamala & Prasad, 1995), for storage of organs in transplantation, to enhance the quality of silver emulsion of photographs, as a carrier for drug stability and distribution (Ulbrich & Subr, 2004) and as a vaccines carrier (Sahoo *et al*., 2007). Conjugation of dextran and inulin is more effective to increase the pharmaceutical utilization of drugs (Molteni, 1985).

Different fermentation technologies such as submerged fermentation, the solid-state fermentation, and surface culture are used for the synthesis of microbial enzymes and metabolites. However, the production of dextransucrase enzyme in submerged fermentation has been more recognized in comparison to solid-state fermentation. This submerged fermentation system is easier to use because of easy to control the process and sterilization (Subramaniyam & Vimala, 2012). Certain factors such as pH, temperature, sucrose concentration, nitrogen concentration, aeration in the medium, agitation speed, incubation time, and medium composition affect the biosynthesis of the dextransucrase enzyme. By the optimization of these factors, dextransucrase activity can be improved (Al-doori *et al*., 2015; Lule *et al*., 2016; Onilude *et al*., 2013). Sucrose is mostly utilized as a single carbon origin for the synthesis of dextransucrase (Dols *et al*., 1997; Vettori *et al*., 2012). So, only sucrose hydrolyzing organisms grow on this media. Also, vancomycin and tetracycline add to the medium for the isolation of dextransucrase-producing bacteria. *Leuconostoc* species are resistant to these antibiotics. By adding $CaCl_2$ in the medium, dextransucrase production is enhanced but in the presence of EDTA, the dextransucrase activity is reduced (Kim, 1992). Moreover, cadmium, copper, mercury, and lead also show inhibitory action towards the dextransucrase enzyme (Robyt & Walseth, 1979). Dextransucrase requires optimum temperature of 25-30°C and a pH of 5.0- 5.5 for its activity. Moreover, this enzyme is highly stable under its optimum conditions which enhances its uses (Naessens *et al.,* 2005). About 33% of glycerol and 0.1% of serum albumin can increase the stability of the dextransucrase enzyme. The activity of dextransucrase can be enhanced with the supplementation of exogenous dextran (Kobayashi *et al.,* 1986).

MATERIALS AND METHODS

Isolation of Dextransucrase Producing Bacteria

For the separation of dextransucrase synthesizing bacteria, sucrose based medium (SBM) comprising (g/L, w/v) sucrose 40, yeast extract 20, K_2HPO_4 20, $MgSO_4$.$7H_2O$ 0.2, $MnSO_4.H_2O$ 0.01, NaCl 0.01, $CaCl_2$ 0.02, $FeSO_4.7H_2O$ 0.01, and agar 18 (pH 6.9) was utilized. The medium was also supplemented with filter-sterilized tetracycline (30 µg/ml) and vancomycin (0.20 µg/ml). Fourteen different bacterial strains were isolated from seven different samples (cucumber, cabbage, eggplant, grapes, milk, pepper and sugarcane). The nutrient agar medium was used to maintain the culture of isolates.

Submerged Fermentation

Inoculum preparation: A 100 ml Erlenmeyer flask comprising 10 ml of sterilized SBM was inoculated with a single cell colony from each freshly streaked plate. Placed all the flasks in a shaking incubator

(180 rpm) for overnight growth at 30°C.

Fermentation technique: Each 250ml Erlenmeyer flask comprising 50 ml of sterilized SBM was inoculated with one percent of overnight grown inoculum and sited in a shaking incubator (180 rpm) at 30°C for 48 hours. After incubation, the culture was centrifuged at 6000 rpm for 15 minutes and the supernatant was utilized for the analysis of enzyme activity.

Dextransucrase Assay

The activity of the dextransucrase enzyme was observed by the process as described by Bounaix *et al*., (2010) and Iliev & Vasileva (2012). For assay 20 mM sodium acetate buffer (pH 5.4) comprising (g/L) sucrose 100, $CaCl_2$ 0.05 and NaN_3 1.0 was prepared. 0.5 ml of the sodium acetate buffer and 0.5 ml of supernatant (enzyme) were transferred in a test tube and incubated in a water bath for 60 minutes at 30°C. After 1 hour, 1 ml of DNS was added into each test tube for the detection of reducing sugar (*Michelena et al., 2003*). Optical density was observed on a UV spectrophotometer at 540 nm. All the experiments were run in duplicate. One unit of dextransucrase is defined as the amount of dextransucrase that releases 1 µmol of reducing sugar (fructose) per minute under standard assay conditions.

Following ten different sucrose-based medium were screened for the formation of dextransucrase.

M1 (g/L): Sucrose 40, Tryptone 10, yeast extract 5, pH 6.9 (Mayeux & Colmer, 1961)
M2 (g/L): Sucrose 40, Peptone 5, Yeast extract 5, K_2HPO_4 15, $CaCl_2$ 0.05, $MnCl_2.H_2O$ 0.01, NaCl 0.01, pH 7 (Sarwat *et al*., 2008)
M3 (g/L): sucrose 40, yeast extract 20, K_2HPO_4 20, $MgSO_4$.$7H_2O$ 0.2, $MnSO_4.H_2O$ 0.01, NaCl 0.01, $CaCl_2$ 0.02, $FeSO_4.7H_2O$ 0.01, pH 6.9 (Dols *et al*., 1997)
M4 (g/L): Sucrose 40, Yeast extract 10, $MgSO_4.7H_2O$ 0.04, $CaCl_2.2H_2O$ 0.14, $FeSO_4.7H_2O$ 0.04, $MnSO_4.H_2O$ 0.02, NaCl 0.01, H_3PO_4 5.7, pH 6.9 (Guzman *et al*., 2018)
M5 (g/L): Sucrose 40, K_2HPO_4 15, Peptone 10, Beef extract 10, yeast extract 5, Sodium acetate 5.0, $MgSO_4.7H_2O$ 0.1, Tween 80 10, $MnSO_4.H_2O$ 0.05, pH 6.7 (Hu & Ganzle, 2018)
M6 (g/L): Sucrose 40, Yeast extract 15, Na_2HPO_4 20, pH 6.7 (Michelena et al., 2003)
M7 (g/L): Sucrose 40, Yeast extract 5, Sodium acetate 5, Peptone 10, tween 80 1, K_2HPO_4 2, $MnSO_4.4H_2O$ 0.01, $MgSO_4.7H_2O$ 0.2, $CaCl_2.2H_2O$ 0.02, pH 6 (Sharma *et al*., 2016)
M8 (g/L): Sucrose 40, Yeast extract 18.5, Beef extract 15.3, pH 6.9 (Purama & Goyal, 2008)
M9 (g/L): Sucrose 40, Tryptone 10, Yeast extract 1.0, K_2HPO_4 2.5, pH 7 (Sarwat *et al*., 2008)
M10 (g/L): Sucrose 40, K_2HPO_4 5, Yeast extract 2.5, $(NH_4)SO_4$ 0.2, NaCl 0.6, $MgSO_4.7H_2O$ 0.2, pH 6.9 (Dimic, 2006).

RESULTS AND DISCUSSION

Isolation and Screening of Bacterial Strains for the Production of Dextransucrase

From the seven different samples of milk, fruits, and vegetables, fourteen different bacterial strains were isolated using SBM. To find the most productive strains for dextransucrase production, the isolates were screened for the biosynthesis of dextransucrase using the shake flask method. Among the fourteen different bacterial isolates, the strain coded as IIB-C9 exhibited highest dextransucrase activity (4.8 U/ml) as shown in Table I. Therefore, this bacterial isolate was further used to attain the highest yield of the enzyme by optimizing the cultural conditions.

Morphological and biochemical characteristics of the bacterial isolate IIB-C9 were also studied. The bacterial strain IIB-C9 was gram-positive cocci. A study of colony morphology showed that the colonies of the isolate were transparent with undulate margins and flat shape. The isolate was catalase and oxidase negative as shown in Table II.

Selection of Medium for the Maximum Production of Dextransucrase

Ten different sucrose-based medium were screened for the synthesis of dextransucrase. Evaluation of different media for enzyme production is shown in Fig. 1. Maximum production of the enzyme (5.34 U/ml) was obtained by the fermentation medium M6, which contained sucrose, Na_2HPO_4, and yeast extract (Michelena *et al.*, 2003). All the other media gave relatively less production of the enzyme.

Effect of pH on the Production of Dextransucrase

The dextransucrase production by the strain IIB-C9 was carried out at different pH to determine optimum pH (Fig. 2). The maximal dextransucrase activity (5.46 U/ml) was observed at a pH of 6.95. Hence the pH 6.95 was selected as the optimal pH for the strain IIB-C9 for the production of dextransucrase. So that isolated bacterial strain could grow best at this

neutral pH. The [H^+] in the environment can affect microbes. A small change in the [H^+] in the medium affects a huge amount of proteins such as enzymes. The pH maintains the ion balance at the active site of the enzyme. When there no optimum pH level is maintained in the culture medium, the 3D arrangement of the amino acids present in the enzyme might be distorted due to the imbalance of ions. Consequently, it results in a loss of enzyme activity (Widowati *et al*., 2017). The outcomes of the current study were in contrast with the results observed by Al-doori *et al.* (2015), in which he observed the maximal dextransucrase production at pH 6.5.

Table I: Screening of bacterial isolates for the production of dextransucrase

Sr. No.	Samples	Strain code	Dextransucrase activity (U/ml)
1.	Milk	IIB-C1	1.8
2.	Milk	IIB-C2	0.9
3.	Grapes	IIB-C3	0.67
4.	Grapes	IIB-C4	0.60
5.	Cabbage	IIB-C5	1.98
6.	Cabbage	IIB-C6	3.12
7.	Eggplant	IIB-C7	1.2
8.	Eggplant	IIB-C8	1.8
9.	Sugarcane	IIB-C9	4.8
10.	Sugarcane	IIB-C10	1.8
11.	Carrot	IIB-C11	2.4
12.	Carrot	IIB-C12	3.0
13.	Pepper	IIB-C13	0.66
14.	Pepper	IIB-C14	1.8

Table II: Morphological and biochemical characters of bacterial strain IIB-C9

Details of The Experiment	Observations/Results
Shape of bacteria	Cocci
Gram character	+ve
Growth	Rapid
Shape	Flat
Margin	Undulate
Color	Transparent
Opacity	Opaque
Catalase	-ve
Oxidase	-ve

Effect of Temperature on the Production of Dextransucrase

To check the impact of temperature on dextransucrase production by the isolate IIB-C9, submerged fermentation was carried out at different temperatures (30, 37, and 45°C) as shown in Fig. 3. At 30°C, the maximal synthesis of dextransucrase (5.52 U/ml) was obtained by the strain IIB-C9. Further increase in temperature decreased the enzyme production. Temperature is a critical factor that affects the production of the enzyme. Too much low temperature causes a decrease in cell metabolism.

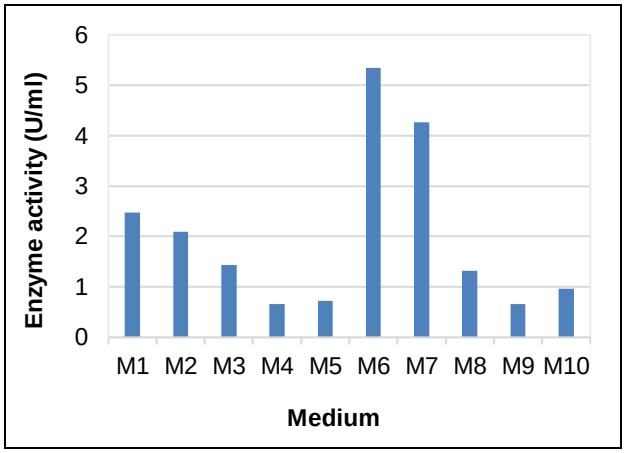

Fig. 1: Selection of medium for the maximum production of dextransucrase

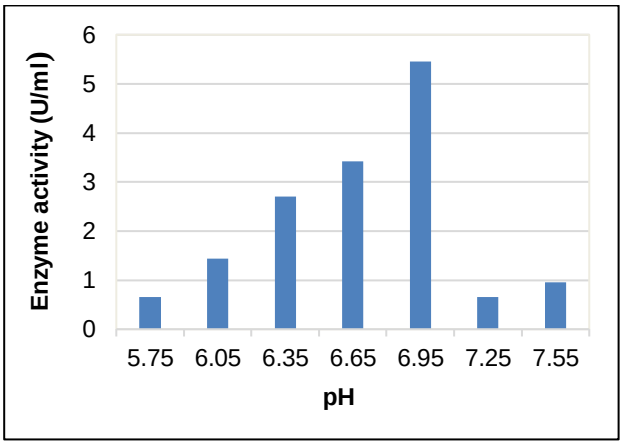

Fig. 2: Effect of pH on the production of dextransucrase

Also, a higher temperature causes the damage of cell proteins and enzymes. Denaturation of enzymes due to high temperature results in the loss of enzyme activity (Widowati *et al.*, 2017). The outcomes of the current study were in accordance with Sarwat *et al*. (2008) as they also observed the maximum dextransucrase activity at 30°C.

Effect of Sucrose Concentration on the Production of Dextransucrase

The effect of different sucrose concentrations on dextransucrase synthesis by strain IIB-C9 was

also examined. The enzyme was produced in different flasks containing optimum medium supplemented with different concentrations of sucrose from 1-6%. Fig. 4 showed the production of dextransucrase enzymes at different sucrose concentrations.

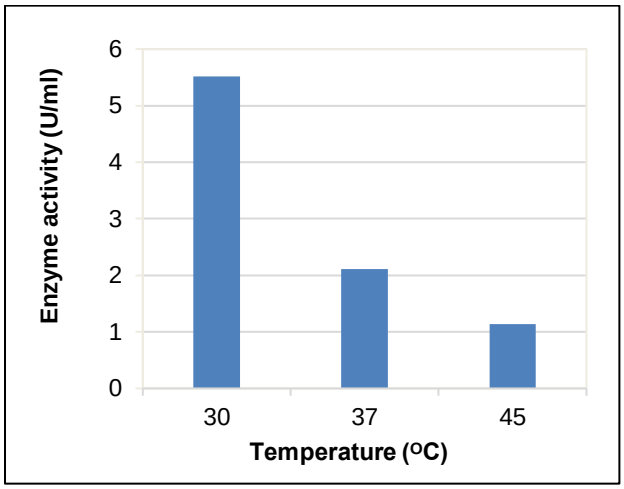

Fig. 3: Effect of temperature on the production of dextransucrase enzyme

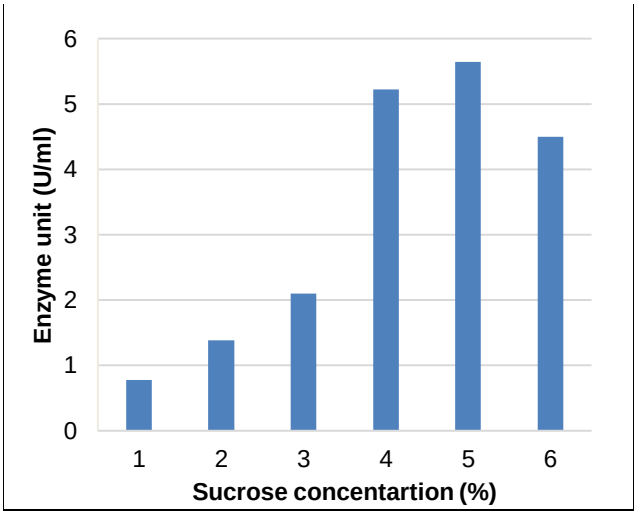

Fig. 4: Effect of sucrose concentration on the production of dextransucrase

The maximum production of dextransucrase activity (5.64 U/ml) was achieved by the strain IIB-C9 when the cultivation medium was supplemented with 5% sucrose. Increasing or decreasing the sucrose concentration beyond this level, causes a reduction in enzyme production. The outcomes of the present study were in contrast with the outcomes observed by Dols *et al.* (1997) in which they observed the maximum dextransucrase production with the 4% sucrose concentration.

Effect of Yeast Extract on the Production of Dextransucrase

The influence of different concentrations of yeast extract (1, 1.5, 2, 2.5, 3, 3.5, and 4%) on dextransucrase production by IIB-C9 was also examined (Fig. 5). Maximal production of dextransucrase (6.12 U/ml) was obtained when the fermentation medium was improved with 2.0% yeast extract. Nitrogen sources act as a secondary energy source for the growth of organisms, which specifically show a significant part in the synthesis of important enzymes (Dutta *et al.*, 2016). The outcomes of the current study were in contrast with the results observed by Dols *et al.* (1997) in which they observed the maximum dextransucrase production with the 4% yeast extract concentration.

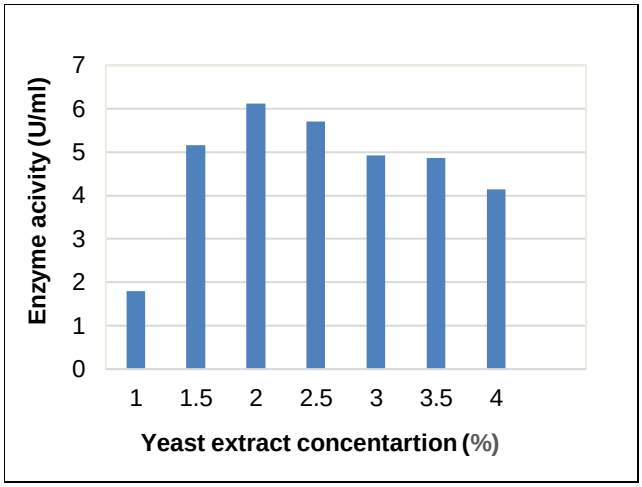

Fig. 5: Effect of yeast extract on the production of dextransucrase

Time Course Study for the Maximum Production of Dextransucrase

The influence of the incubation period on dextransucrase production by the strain IIB-C9 was also examined in the present work. Fig. 6 depicts the dextransucrase activity at altered incubation periods (24, 48, 72, and 96 hours) by the strain IIB-C9. At the incubation period of 24 hours, the maximum dextransucrase activity (9.96 U/ml) was obtained. Further increase in the incubation period caused a declined in the enzyme production. It might be because of the exhaustion of nutrients in the medium. When the nutrients deplete from the culture medium, the bacterial cells enter a stationary phase, where secondary metabolites are produced. These

secondary metabolites may interfere with the production of the enzyme (Tiwari *et al*., 2014). The outcomes of the current study were in contrast with the outcomes of Purma & Goyal (2009), who reported maximum enzyme production at the incubation temperature of 25 hours.

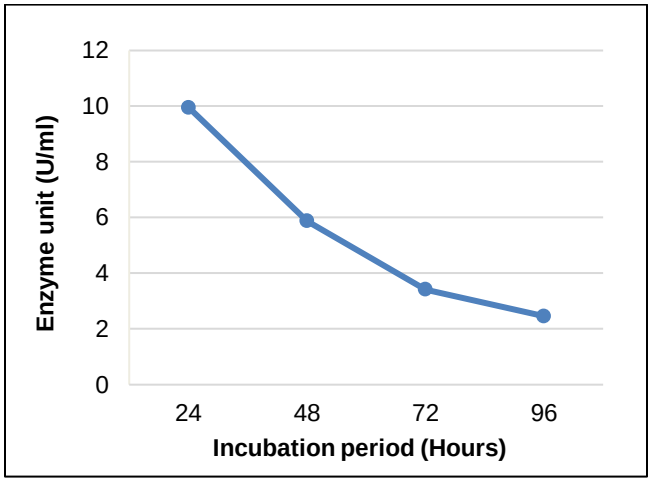

Fig. 6: Time course study for the production of dextransucrase

CONCLUSION

In the current study, a submerged fermentation technique was used for the synthesis of dextransucrase from the bacterial strain IIB-C9. The enzyme production was optimum when submerged fermentation was carried out using medium M6 containing (g/L) sucrose 50, Na_2HPO_4 20 and yeast extract 20), initial pH 6.95, temperature 30°C, and incubation period 24 hours. However further parametric optimization is still needed to improve the production of dextransucrase before pilot-scale studies.

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