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STUDY OF DIFFERENT FACTORS EFFECTED IN PRODUCTION OF DEXTRANASE ENZYME FROM A LOCAL ISOLATE OF *B. SUBTILIS* Z2 BACTERIA

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Abstract

The present study aims to detection optimal conditions of production of dextranase enzyme from isolate of *B. subtilis* Z2. Eight carbonic sources were represented by Dextran, Molasses, fructose, sucrose, glucose, Lactose, CMC and Arabinose) at concentration of 0.5% for each source. It was found that the best was represented by Molasses carbonic which showed higher activity and qualitative activity of 7.11 units / ml and 83.01 units / mg. The nitrogen sources were selected, including yeast extract, peptone, yeast extract with peptone, gelatin, meat extract, Ammonium sulphate, Potassium nitrate and Ammonium chloride. These sources were added at a concentration of 0.5% to the production medium. The Peptone with yeast extract source was the best of used nitrogenic sources at concentration of 1.2%, which showed higher activity and qualitative activity at 8.89 units / ml and 85.5 units / mg. The best hydrogen number was 7. The size of inoculums of 3 × 107 c.f.u. was the best which showed higher activity and qualitative activity of 21.702 units/ ml and 301.29 units/ mg. there incubation which 44 hours showed higher activity and qualitative activity of 32.934 units / ml and 633.52 units / mg.

Key words: dextranase enzyme, *B. subtilis*, carbonic sources, nitrogen sources.

Introduction

Enzymes are biological catalysts; they are highly specialized catalytic proteins with extraordinary catalytic power and also have remarkable specificity. They are essential for all forms of life by catalyzing the various chemical reactions in the cells. Even though enzymes catalyze only one kind of chemical reaction, there are many enzymes in a typical cell. Enzymes are soluble and colloidal substances characterized by great activity and specificity. They have their core roles in survival, growth and metabolism in living systems (references please) Dextranase [(6-1)-α-D-glucan6-glucanohydrolases or glucanases (E.C.3.2.1.11)]. The enzyme is a specialist for hydrolysis of α-1-6 in dextran.

Dextran hydrolysis products by dextranes are glucose, isomaltose and isomalto-polysaccharides. Source (Jaiswal and Kumar, 2011), Dextranases are enzymes that break down high molecular weight dextran molecules into molecules of lower molecular weight and thus reduce the viscosity of molasses. The use of dextranes stops the production of dextran (dextran formation), reduces the boiling time and the product flows smoother due to low viscosity. (Cuddihy *et al*., 2000; Jiao *et al*., 2014). The presence of dextranase during the early stages of polysaccharide production inhibits the formation of insoluble polymer in water produced by *Streptococcus mutans* by Dextransucrase and thus inhibits the onset of α-1-6 synthesis. (Walker, 1972), The use of dextranase is the most effective method of dextran decomposition in sugar factories. Some of these dextranases have been developed in enzymatic preparations and have been used efficiently for the decomposition of dextran in sugar mill juices (Arya *et al*., 2018). Industrially important enzymes have traditionally obtained from submerged fermentation (SMF) because of the ease of handling and greater control of environmental factors such as temperature and pH (reference please). The use of submerged culture is advantageous because of the easy of sterilization and process control is easier to engineer in these systems. Depending upon on the strain and culture conditions, the enzyme can be cultivable or inducible showing different production patterns. The purpose of this work was to study the production of dextranase by *Bacillus subtilis* Z2 in submerged fermentation and optimized culture conditions for the production of amylase (references please).

Materials and Methods

Superoxide dismutase

Was followed the method described by Whitaker and Bernard, (1972); Lin *et al*., (1997) 7\7estimating the effectiveness of the enzyme Dextranase and based on reduced sugars released as a result of hydrolysis of the dextran by the enzyme. The enzyme activity unit (unit / milliliter) is defined as the amount of enzyme released (1) micromolecule of sugars (glucose) per min and under conditions of estimation or measurement.

Determination of enzyme concentration

Determination of protein concentration performed according to Bradford, (1976).

Determination of optimal conditions for enzyme production

The effect of a number of environmental factors were studied to determine the optimum conditions for the production of the enzyme from selected bacterial isolate, which include: concentration of carbon source, nitrogen source in the production medium, primary pH, temperature and incubation period.

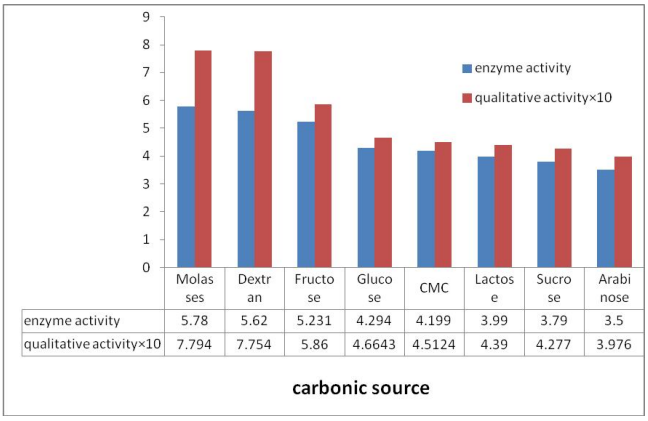
Determination of optimal carbon sources for enzyme production

Various carbon sources (Glucose, Molasses, Fructose, Dextran, Lactose, Sucrose, CMC, Arabinose) were selected with a concentration of 0.5% to determine the optimal carbon source for the enzyme production under study as well as to study the effect of different concentrations from the optimum carbonate source to the liquid production medium with concentrations of 0.5, 1.0, 1.5, 0.2, 2.5,3.0 and 3.5%.

Determination of the optimal nitrogen sources for enzyme production

Various nitrogen sources were used to determine the optimal nitrogen source for the production of the enzyme amylase from the isolation. The types of nitrogenic sources were selected: Peptone, Yeast extract, Gelatin, yeast extract with peptone, meat extract, ammonium chloride (NH_4Cl), potassium nitrate (KNO_3) and ammonium sulphate $(NH_4)_2\ SO_4$.

These sources were added to the production medium at a concentration of 0.5%. it was also studied the effect of different concentrations of the optimal nitrogen source for the production of the enzyme by adding the selected nitrogen source to the liquid production medium with different concentrations (0.3, 0.6, 0.9, 1, 1.2, 1.5 and 1.8).



	Molasses	Dextran	Fructose	Glucose	CMC	Lactose	Sucrose	Arabinose
enzyme activity	5.78	5.62	5.231	4.294	4.199	3.99	3.79	3.5
qualitative activity×10	7.794	7.754	5.86	4.6643	4.5124	4.39	4.277	3.976

Fig. 1: Effect of carbonic sources at 0.5% concentration on the production of Dextranase enzyme from isolate *B. subtilis* Z2.

Selection of optimum primary pH for the production of dextranase enzyme

The optimal production medium for the production of the enzyme under study was observed from different pH numbers (5, 5.5, 6, 6.5, 7, 7.5 and 8) and incubators containing the isolates were inculcated after Hydrogen is optimized to produce the enzyme dextranase.

Optimization of temperature to produce the enzyme dextranase

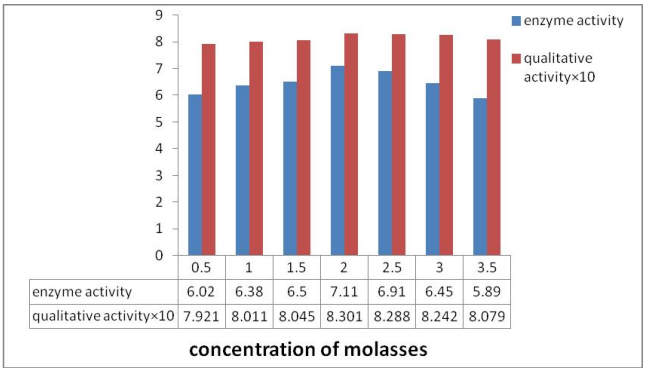
Bacterial cells were inoculated at different temperature (25-55°C) for 96 hours, incubation was established to determine the optimum temperature for the production of the enzyme.

Determination the size of the inoculates to optimize the enzyme production

The production of the enzyme from isolate under study was followed by modification of the components of the medium and production conditions according to the results obtained from the previous experiments, where the circles were inoculated with the size of the inoculate (1, 2, 3, 4, 5, 6, 7) × 10^7 cells.

Determination of optimal incubation periods to produce dextranase enzyme

The vials containing the optimal production medium



	0.5	1	1.5	2	2.5	3	3.5
enzyme activity	6.02	6.38	6.5	7.11	6.91	6.45	5.89
qualitative activity×10	7.921	8.011	8.045	8.301	8.288	8.242	8.079

Fig. 2: Effect of different concentrations of molasses on the production of Dextranase enzyme from isolate *B. subtilis* Z2.

after incubation with bacterial cells for isolation under study were incubated at a different time periods (12, 20, 28, 36, 44, 52 and 60) at 35°C. The enzyme efficacy were estimated at the end of each incubation period to produce dextranase.

Results and discussion

Determine the optimal carbon source to produce the enzyme

Nine carbon sources were selected to study its effected in the production of dextranase, dextran, molasses, fructose, sucrose, glucose, arabinose, lactose and CMC, with 0.5 % concentration of each source. The highest enzyme production of *B. subtilis* Z2 was showed within 24 hours of incubation at 35 m, in terms of enzymatic activity and quality 5.78 units / ml and 77.94 units / mg with molasses as a source of carbon followed by dextran with enzymatic efficiency and quality of 5.62 units / ml and 77.54 Unit / mg, glucose is effectively enzyme of quality 4.294 units / ml and 46.643 units / mg and fructose effectively enzymatic and 5.231 quality unit / ml and 58.6 units / mg and CMC effectively enzymatic quality 4.199 units / ml and 45.124 units / mg, then lactose, sucrose and arabinose respectively.

The researcher (Shahid *et al*., 2019) used different sources of carbon where glucose, cellulose, starch, xylan, pectin, maltose, dextran 5 kDa, dextran 10 kDa, dextran 270 kDa of *Bacillus megaterium* were the highest enzymatic activity obtained When dextran 10 kDa was used as a carbon source, it was incubated at 50°C for 24 hours. In another study, the effect of five carbon sources to produce dextranase enzyme from *Bacillus subtilis* NRC-B233 was corn flour, wheat flour and wheat bran. The best source of carbon was flour The enzyme efficiency was 75.276 IU / ml using the incubator and the enzymatic activity 61.323 using the static incubator at a temperature of 30°C for 24 hours (Mansour *et al*., 2011). (Mahmoud *et al*., 2014) also studied the effect of different carbon sources to produce dextranase. Of *Penicillium aculeatum* NRRL-896 namely dextran, glucose, lactose, dextrin, starch was the best source for the production of enzyme the dextran.

It was noted that the best concentration of Molasses was 2% in terms of enzymatic and quality activity, which amounted to 7.11 units / ml and 83.01 units / mg respectively. was found as in the following figure.

Determine the source of the optimum nitrogen to produce the enzyme

Several organic and an organic nitrogen sources were selected to investigate their effect on the production of dextranase from isolation under study. Yeast extract, peptone, Yeast extract with peptone, gelatin, meat extract, ammonium sulphate $(NH_4)_2SO_4$, potassium nitrate KNO_3 and ammonium chloride NH_4Cl. These sources were added at a concentration of 0.5% to the production medium. The results in fig. 3, showed that Yeast extract with peptone was the most efficient in the production of dextranase compared to the other nitrogen sources. The enzymatic and qualitative activity reached were 7.11 units / ml and 83.01 units / mg.

This result is consistent with the findings of the researcher (Zohra *et al*., 2013) where it stated that the best nitrogen source for the production of enzyme dextranase from *Bacillus licheniformis* bacteria is peptone and yeast extract together where the researcher used the yeast extract and tryptone and peptone separately and peptone and yeast extract together, the researcher (Mouafi *et al*., 2016) found that the optimal nitrogen source for the production of dextranase from *Aspergillus awamori* F-234 based on enzymatic and specific efficacy is ammonium nitrate compared to ammonium oxalate, ammonium sulphate, urea and

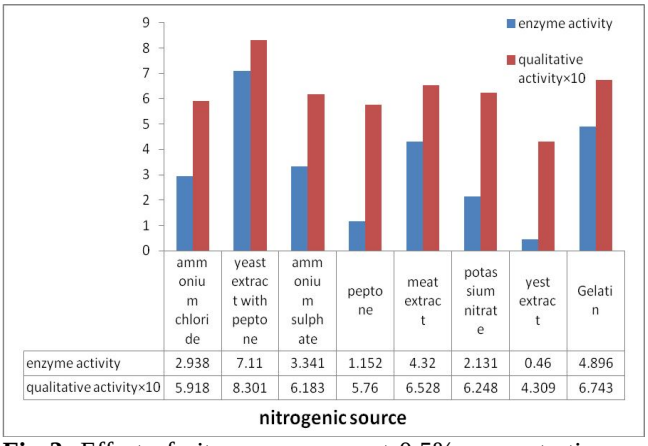


Fig. 3: Effect of nitrogen sources at 0.5% concentration on the production of the enzyme Dextranase of isolate *B. subtilis* Z2.

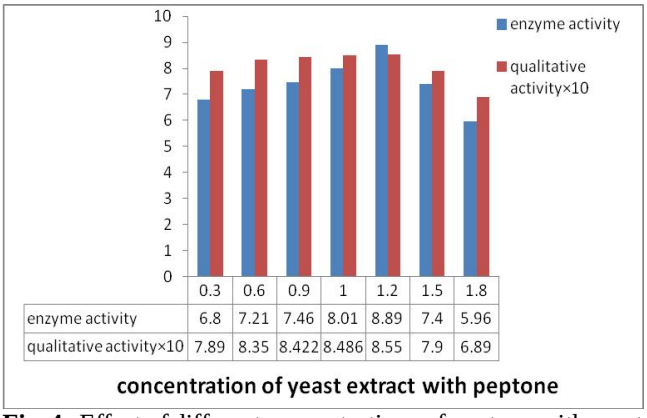


Fig. 4: Effect of different concentrations of peptone with yeast extract on the production of Dextranase enzyme from isolate *B. subtilis* Z2.

diphosphate. It has given the highest productivity Of dextranase when grown in a medium containing tryptophan as a source of nitrogen compared to lysine and kleicin for 48 hours (Purushe *et al*., 2012), as researcher (Esawy *et al*., 2012) reported that the best dextranase production from *Bacillus subtilis* NRC-B233b Peptone as a nitrogen source as compared to other nitrogen sources such as casein, ammonium chloride, yeast extract, urea, beef extract and corn steep. found that the best concentration of peptone with yeast extract was 1.2% For enzyme production of *B.subtilis* Z2.

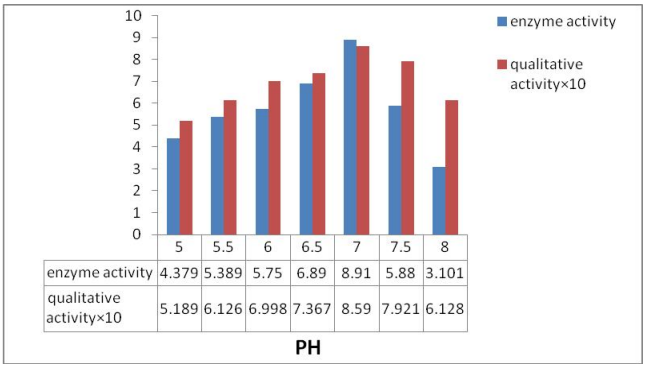
Determination of the optimal pH for enzyme production

The local isolate ability to dextranase production with different pH values ranging from (5-8) and half a degree from one center to another was tested. The increment in enzyme production was observed by increasing in the primary pH of the medium by mean of enzymatic efficiency and quality and reaching a maximum of 8.91 units / ml and 85.9 units / mg respectively, as shown in fig. 5 at pH 7.0 and then gradually decreased at basal values of pH.

The researcher (Mouafi *et al*., 2016) found that the best pH for dextranase production from *Aspergillus awamori* F-234 was 6.0 in terms of efficacy and specificity, while the researcher (Wang *et al*., 2014) found that the optimal pH for dextranase production from *Arthrobacter* KQ11 It is 5.5 and the researcher (Cai *et al*., 2013), confirmed that the best pH to produce the enzyme from the bacterium *Catenovulum sp*. was 8.0.

Set the optimal temperature to produce the enzyme

The effect of a range temperatures in the production of dextranase was ranged between (25-55°C). The results in fig. 6, showed a decrease Enzyme production at 25°C. The isolation under study gave enzymatic quality and quality of 8.51 units / ml and 85.03 units / mg respectively. This activity increased to a maximum of 35°C with enzymatic activity and quality of 8.93 units / ml and 86.01 mg / Unit / mg respectively and a decrease in productivity was observed at a temperature above 35°C, indicating that the temperature 35°C was the optimal for the production of isolation under study. The researcher (Bhatia *et al*., 2015) studied the effect of cuddling temperature on the production of the enzyme from *Paecilomyces lilacinus* and the optimal degree of production of the enzyme is 30°C, while the researcher (Wakil *et al*., 2018) found that the optimal production of dextranes enzyme from *Penicillium brevicompactum* was at The temperature of 30°C, (Subasioglu *et al*., 2010), found that the best production of dextranes was at 30°C of *Paecilomyces lilacinus*.



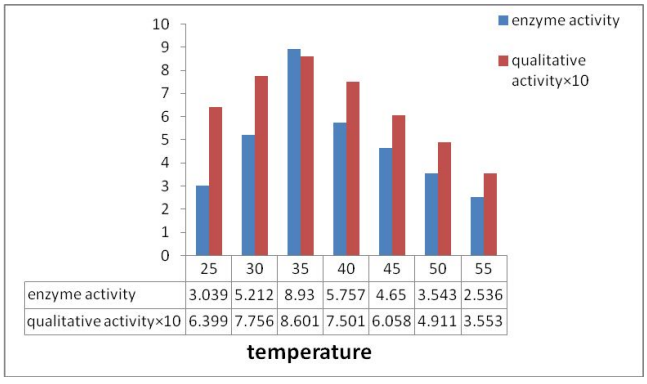
	5	5.5	6	6.5	7	7.5	8
enzyme activity	4.379	5.389	5.75	6.89	8.91	5.88	3.101
qualitative activity×10	5.189	6.126	6.998	7.367	8.59	7.921	6.128

Fig. 5: Effect of pH in the production of Dextranase enzyme from isolate *B. subtilis* Z2.

Determination of optimal inoculums volume enzyme

Different sizes of the inoculums were used to inoculate the production medium as shown in the fig. below. It was found that there was a gradual increase in the production of the enzyme with the increase in the size of the vaccine added to the production medium in terms of enzymatic activity and specific effectiveness With a maximum of 21.702 units / ml and 301.29 units / mg at the addition of 3×10^7 c.f.u but significantly decreased with enzymatic efficacy and efficacy of 6.11 units / ml and 108.5 units / mg when using a vaccine size of 7×10^7 c.f.u. These results obtained showed that the size of the high vaccine does not necessarily produce high production of the enzyme, but may result from the high size of the vaccine for nutrient intake in the early plant medium as well as the depletion of oxygen due to the rapid growth of the farm and the aggregation of cells, Oxygen and the proportion of nutrient consumers thus reduced enzyme productivity (Haritha *et al*., 2011; Muthulakshmi *et al*., 2011; Patil and Chaudhari, 2013).

The optimal productivity of the enzyme from *Paecilomyces lilacinus* was (Bhatia *et al*., 2015) when the vaccine size was used 1×10^7 cells / ml at a temperature of 30°C and pH 6.0, while the researcher found (Shahid *et al*., 2019). The optimal production of the enzyme



	25	30	35	40	45	50	55
enzyme activity	3.039	5.212	8.93	5.757	4.65	3.543	2.536
qualitative activity×10	6.399	7.756	8.601	7.501	6.058	4.911	3.553

Fig. 6: Effect of different temperature in the production of Dextranase enzyme from isolate *B. subtilis* Z2.

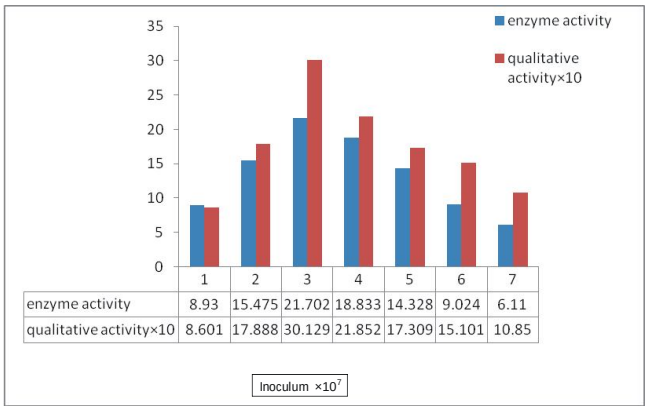


Fig. 7: Effect of vaccine volume in the production of Dextranase enzyme from isolate *B. subtilis* Z2.

dextranase from *Bacillus megaterium* requires the size of the vaccine 2×10^8, while the researcher (Alili, 2001) that the optimal production of the enzyme from the bacterium *Sterptomyces sp* AM when using the size of the vaccine 8% in terms of effectiveness and specificity and solid-state fermentation method.

Determination of the optimal incubation duration for enzyme production

Test the production of dextranase from the isolate of *B. subtillus* Z2 by inoculation in the center of optimal production and incubation at 35°C and follow the production of enzyme in terms of enzymatic and quality activity at different time periods (12, 20, 28, 36, 44, 52 and 60). The results showed that enzyme production was low after 12 hours of incubation. The enzymatic and enzymatic activity of the enzyme was 21.7 units / ml and 301.13 units / mg, respectively, as shown in fig. 8. An increase in production was observed consistently in the lap and the highest production of the enzyme after 44 hours in terms of enzymatic and quality, which amounted to 32.934 units / ml and 633.52 units / mg respectively and there was a decline in production after 60 hours of lap. Indicating that the optimal incubation period of the enzyme was 44 hours of isolation under study. The gradual increase in the production of the enzyme was observed, with a maximum of 44 hours of incubation.

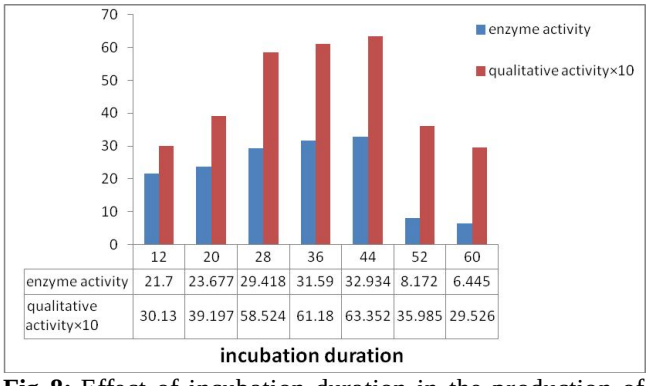


Fig. 8: Effect of incubation duration in the production of Dextranase enzyme from isolate of *B. subtilis* Z2.

Reduced efficacy by increasing the incubation period can be attributed to the fact that only a fraction of the enzyme produced by the bacteria may be degraded either by changes in the medium of production that caused a change in culture conditions due to metabolic products as the bacteria continue to grow or because of the secretion of enzymes. Other proteins from the same bacteria to the production medium include proteolytic enzymes that may have decomposed part of the dextranase molecules in the medium or the depletion of the components of the medium during the incubation period, which adversely affects production (Lazazzera, 2000).

Some studies concerned the lap periods for the production of the enzyme according to the production conditions and the components of the medium and the type of microorganism. The researcher (Subasioglu and Cansunar, 2010) studied the production of dextranase enzyme from *Paecilomyces lilacinus*. (1, 2, 3, 4, 5, 6, 7, 8, 9) day and the duration of 7 days is optimal in terms of specificity and biomass and the researcher (Mouafi *et al*., 2016) concluded that the production of dextranase enzyme from *Aspergillus awamori* F-234 as he studied different periods (1, 2, 3, 4, 5, 6, 7) days. The optimum duration was 3 days in terms of effectiveness and specificity, reaching 1197 units / ml and 52 units / mg, respectively. (Shahid *et al*., 2019) used different incubation periods (6, 12, 18, 24, 48, 72, 96) hours to produce dextranes in *Bacillus megaterium* and the best production period was 24 hours in terms of enzymatic activity which was approximately 450 units / ml.

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