

Optimization and characterization of dextransucrase production by local *Leuconostoc mesenteroides*

Asmaa Sabah Al-doori* Aswan Hamdullah Aboud* Ziad TariQ Sedrah**

*Baghdad university/College of Agriculture/Food Science Dep.

** Diyala university/College of Agriculture/Food Science Dep.

Abstract

The dextransucrase production by local *Leuconostoc mesenteroides* was optimized and characterized to reach the optimum conditions of producing this enzyme which used in many food applications such as dextran production. The optimum conditions were using sugar as a carbon source with 20% concentration for giving the highest specific activity of dextransucrase, yeast extract as a nitrogen source, 25°C as incubation temperature, 6.5 optimum production pH and 10⁶ inoculum size. The crude enzyme was characterized for pH stability and heat stability, they were 4-5 and 30°C for an hour, respectively.

The enzyme was purified by using sephadex G-100 with a fold of 4.56 and overall yield of 20.5% of activity, then precipitate by ethanol with a fold of 17.88 and overall yield of 20.64% of activity.

Key Words: Dextransucrase, *Leuconostoc mesenteroides*, Optimization, Characterization.

Introduction

Dextransucrase (E. C. 2.4.1.5) from *Leuconostoc* species are used in industry for the production of oligosaccharides and dextran. Because of their desirable physiochemical properties in food and their prebiotics effect on intestinal bacteria, these oligosaccharides are very useful as food additives. Oligosaccharides produced by dextransucrase find several applications in food, feed, pharmaceutical, or cosmetic industries (5). Dextransucrase (glucansucrase or glucosyltransferase) is an extracellular enzyme that synthesizes dextran from sucrose (3). Many authors have described the effects of sucrose concentration, aeration rate, agitation speed, medium pH, incubation temperature, nature of the yeast extract and other nutritional requirements on the production of dextransucrase, dextran and fructose from sucrose using different strains of *Leuconostoc mesenteroides* (3,5). This enzyme is produced by many varieties of lactic acid bacteria, *Leuconostoc* and *Streptococcus* (6) *Pediococcus*, another lactic acid bacterium may also produce dextransucrase (9). Many authors purified this enzyme by different methods, The results confirmed the multimeric entity of this enzyme, which remains in single molecular form in the native state and separates into multiple bands when denatured by boiling, SDS or 2-mercaptoethanol (8). The aim of this study is to optimize the growth conditions for dextransucrase production by a local isolate of *Leuconostoc mesenteroides* from fermented cabbage.

Material and methods

Many effected factors in dextransucrase production were studied, which include carbon source and its percentages, nitrogen source, pH, temperature, to determine the optimal conditions for dextransucrase production.

Production media: The base media was as follows:

The Substance	The weight: gm/100 ml. D. W.
Sucrose	10
Yeast Extract	0.5
Peptone	0.5
K ₂ HPO ₄	1.5
NaCl	0.001
MgSO ₄ .7H ₂ O	0.001
MnCl ₂ . H ₂ O	0.001
CaCl ₂	0.01

The pH was adjusted to 7.2 before sterilization by autoclave at 121⁰C for 15 minutes.

The changes in this media were done as the optimal results respectively.

Optimal Carbon source:

Many carbon sources were used, whey, maltose, fructose, glucose, sugar, sucrose, malt and date syrup.

Optimal nitrogen source:

Ammonium sulphate, Sodium nitrate, peptone, tryptone, ammonium chloride, ammonium nitrate, yeast extract and thermal yeast extract were used as nitrogen sources.

Optimal temperature:

Temperature degrees (10, 25, 30, 35, 40)⁰c were chosen to determine the optimal temperature for dextransucrase production.

Optimal pH:

Five pH values (5, 6, 6.5, 7 and 8) of fermentation media were determined to stand on the optimal pH for dextransucrase production.

Optimal inoculum's size:

Six inoculum's size of *Leuconostoc mesenteroides* (103, 104, 105, 106, 107,108) were used to emphasize the optimal size for dextransucrase production.

Dextransucrase assay:

Dextransucrase activity was measured according to Michelena et al. method (4) by using sucrose (1.25mg/ml) as a substrate at pH= 5 (0.1M Buffer) for 30 min. at 30⁰c., the reaction was stopped and detected by DNSA reagent.

The enzyme unit was defined as the amount of enzyme that could alter 1 mg of sucrose to dextran and fructose during one hour under test circumstances.

Crude enzyme properties:

1. pH stability:

Crude enzyme was incubated with different pH (3, 4, 4.5, 5, 5.5, 6, 7, 8) For 1 hour, cooled with ice, then the activity was determined.

2. Heat stability:

Crude enzyme was incubated with different temp. (20, 30, 40, 50, 60, 70, 80)⁰c at pH stability value for 1 hour, cooled with ice, the activity was estimated.

Purification of enzyme:

Gel filtration

The first step of purification was gel filtration with sephadex G-100 by using phosphate buffer 0.1M, the enzyme was gained by using phosphate buffer 0.1M, pH=5 containing 0.05M NaCl. The protein was determined by spectrophotometer at 280nm, enzyme activity was determined.

Ethanol participation: the enzyme was participated by cooled ethanol, dextransucrase activity was determined by Michelena et. al. method (4) for the participate part, total activity was determined also. Protein was determined according to a modified method of Lowry (2).

Results and discussion:

From fig.1 we can notice that the best carbon source was sugar for the highest activity and specific activity obtained with 20% concentrate (fig.2). Yeast extract recorded as a best nitrogen source as shown in figure 3. The optimal temperature for enzyme production was 25⁰c, and the optimal pH was 6.5 for the highest activity and specific activity (Fig.4 and Fig.5). The most suitable inoculum size for enzyme production was 106 as shown in figure 6.

These results agreed with Bivolarski et al(1) when they studied the cell wall-bound of the bacteria by using different media, they found the best media for growth and enzyme production is by using glucose and fructose together.

The pH stability of the enzyme was determined at different pH value, it was stable at 4-5 (fig.8). The thermal stability of the enzyme was examined by assaying retained activities after incubation at various temperatures. The residual activity was stable after incubation for 1 hour at temperatures up to 30⁰C.(fig.9). These results were near to what Young-mo et. al.(11) gained of results. Uqader(10) said that the enzyme produced by *Leuconostoc mesenteroides* PCIR-4 was stable at 30⁰C for 240 hrs with 64.2 DSU/ml/hr. and the enzyme was most active in the pH range of 4.75 to 6.0. These results are so close to ours.

The purification step of gel filtration showed that there are 2 narrow peaks and a wide peak of protein, and there is only one sharp peak of dextranase enzyme (fig. 7), in another hand we can see the purification table in table 1 which showed that gel filtration step purified the enzyme with a fold of 4.56 and overall yield of 20.5% of activity and in the second step the enzyme retained his activity at the same yield. This is agreeing with the searcher Purama and Goyal (7).

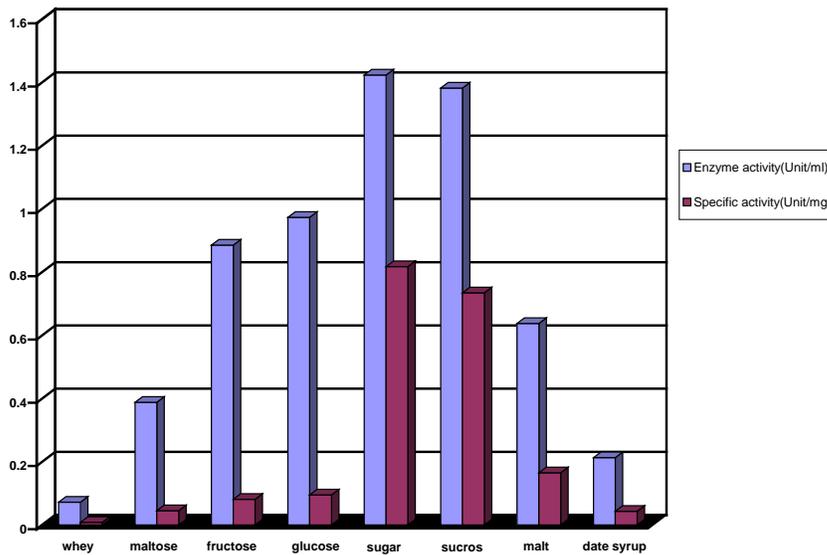


Fig.1 Dextranase activity and specific activity by using different carbon sources

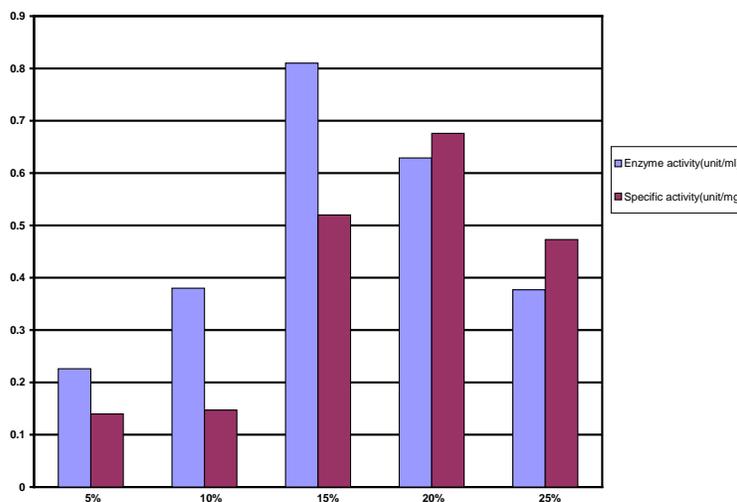


Fig.2 Dextranase activity and specific activity by using different Concentrations of sugar

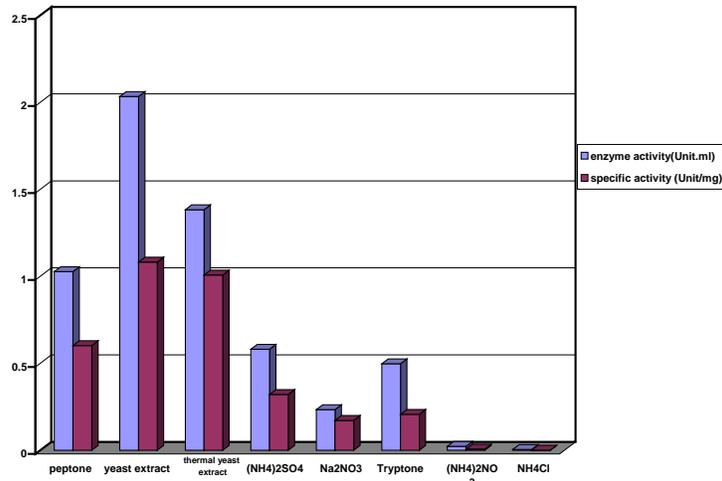


Fig.3 Dextranase activity and specific activity by using different Nitrogen sources

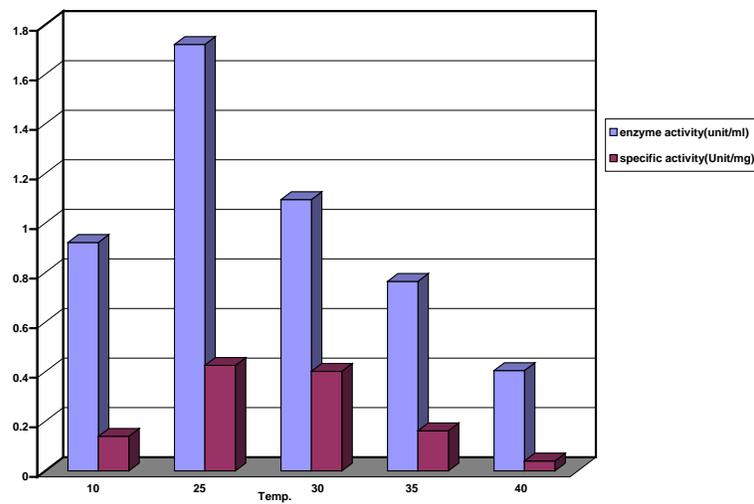


Fig.4 Dextranase activity and specific activity by using different degrees of temperature.

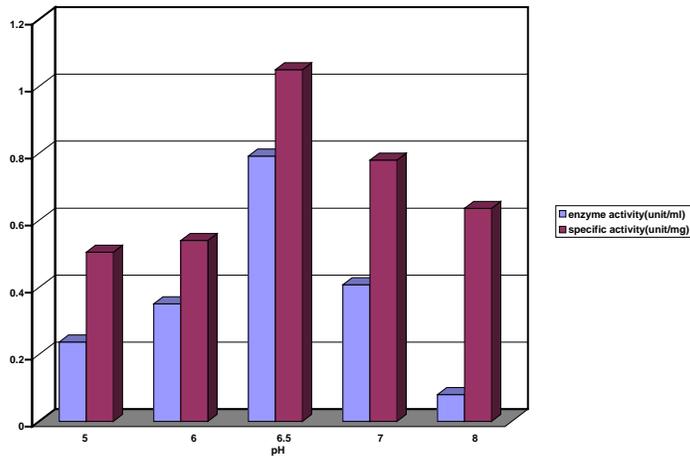


Fig.5 Dextranucrase activity and specific activity by using different pH

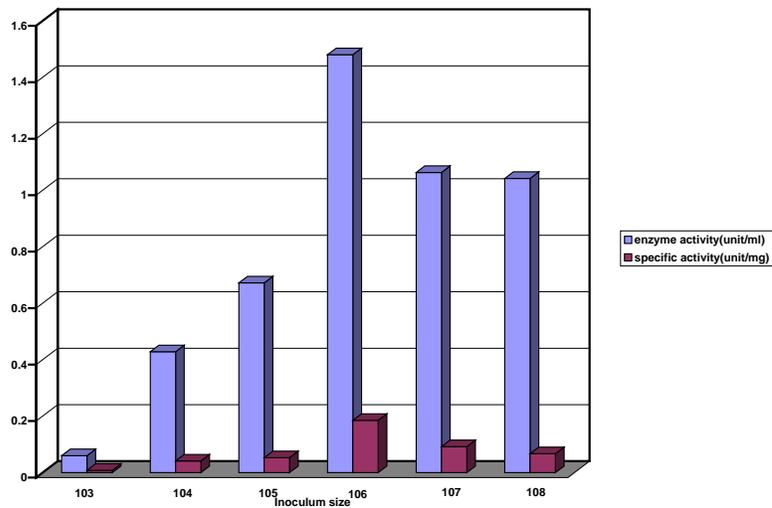


Fig.6 Dextranucrase activity and specific activity by using different Inoculum size

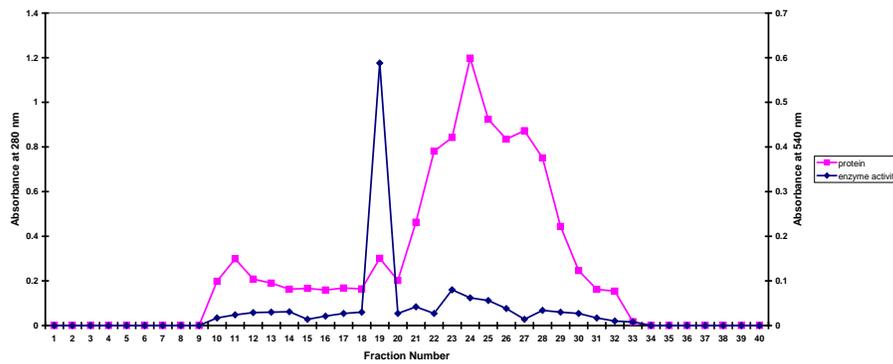


Fig.7 Gel filtration of dextransucrase by Sephadex G-100 with phosphate buffer 0.1M, pH=5 containing 0.05M NaCl.

Table 1. Partial purification steps of dextransucrase

Step of purification	volume	Activity U/ml	Protein mg/ml	Qualitative activity U/mg	Total activity U	Folds of purification	Yields%
Crude enzyme	100	0.218	0.467	0.466	21.8	1	100
Gel filtration	30	0.149	0.070	2.128	4.47	4.56	20.5
Ethanol	3	1.5	0.18	8.333	4.5	17.88	20.64

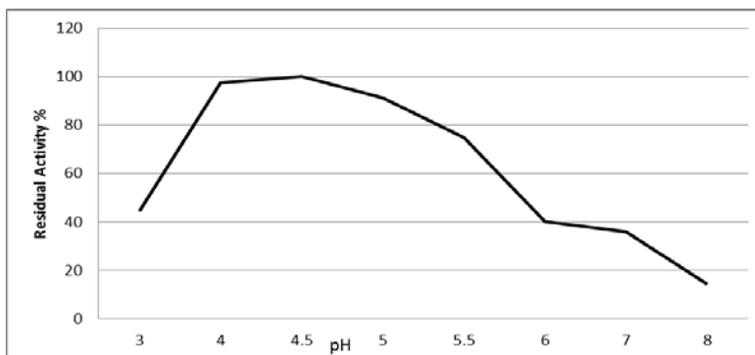


Fig.8 The pH stability of dextransucrase

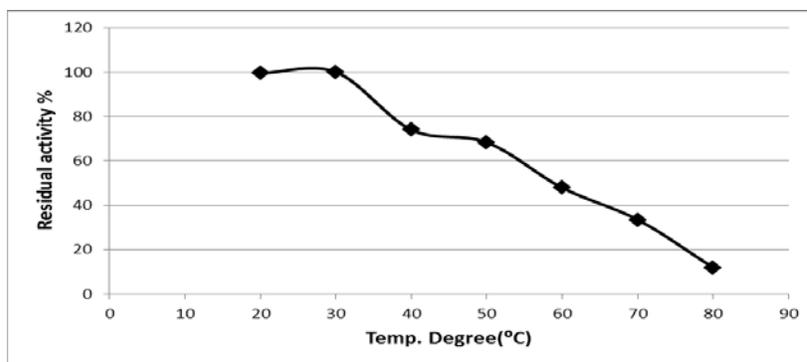


Fig.9 Heat stability of dextransucrase

References:

- [1] Bivolarski, V., Vasileva, T., Dzhambazov, B., Momchilova, A., Chobert, J., Ivanova, I. and Iliev, I. 2013. Characterization of glucansucrase and fructansucrase produced by wild strains *Leuconostoc mesenteroides* URE13 and *Leuconostoc mesenteroides* M17 grown on glucose or fructose as a sole carbon source.
- [2] Cooper, T.G. (1977). The Tools of Biochemistry. A Wiley-Inter Science Publication by John Wiley & Sons, Inc.
- [3] Mary Helen Palmuti Braga Vettori, Cristian Jaques Boner de Lima, Kate Cristina Blanco, Mariana Cortezi, Jonas Contiero. 2012. Performance of response surface model for increase of dextransucrase production by *Leuconostoc mesenteroides* FT 045B under different experimental conditions. Asian Journal of Biological and Life Sciences, vol. 1, issue 1, pp.39-35.
- [4] Michelena, G.L., Martinez, A., Bell, A., Carrera, E. and Valencia, R. 2003. Scale-up of dextransucrase production by *Leuconostoc mesenteroides* in fed-batch fermentation. Brazilian Archives of Biology and Technology, 46(3):455-459.
- [5] Naessens, M., A. Cerdobek, W. Soetaert and E.J. Vandamme, 2005. *Leuconostoc* dextransucrase and dextran: production, properties and applications. J. Chem. Technol. Biotechnol., 80: 845-860.
- [6] Purama, R.K., Goyal, A. 2008. Effect of nutrients by one variable at a time (OVAT) approach on the dextransucrase production from *Leuconostoc mesenteroides* NRRL B-640. Int. J. Microbiol., 5: 1.
- [7] Purama, V. K. and Goyal, A. 2008. Identification, effective purification and functional characterization of dextransucrase from *Leuconostoc mesenteroides* NRRL B-640. Bioresource Technology, Volume 99, Issue 9, Pages 3635–3642.
- [8] Seema Patel, Damini Kothari and Arun Goyal. 2011. Purification and Characterization of an Extracellular Dextransucrase from *Pediococcus pentosaceus* Isolated from the Soil of North East India. Food Technol. Biotechnol. 49 (3) 297–303.
- [9] Smitinont, T., Tansakul, C., Tanasupawat, S., Keeratipibul, S., Navarini, L., Bosco, M., Crescutti, P. 1999. Exopolysaccharide-producing lactic acid bacteria strains from traditional Thai fermented foods: Isolation, identification and exopolysaccharide characterization, Int. J. Food Microbiol., 51: 105–111. [9]
- [10] UIQader, Suha Ali. 2005. Immobilization of dextransucrase for the commercial production of dextran from *Leuconostoc mesenteroides*. Doctor of philosophy thesis, University of Karachi, Pakistan. [10]
- [11] Yong-Mo Kim, Min-Ji Yeon, Nack-Shick Choi, Young-Hyo Chang, Min Young Jung, Jae-Jun Song, Joong-Su Kim. 2010. Purification and characterization of a novel glucansucrase from *Leuconostoc lactis* EG001. Microbiological Research 165, 384–391.