Research & Reviews in Biotechnology & Biosciences *ISSN: 2321-8681*
Volume – 1, Issue – 1, Year – 2014, PP – 33-36

POST HARVEST DETERIORATION OF SUGARCANE JUICE BY DEXTRANSUCRASE ISOLATED FROM *LEUCONOSTOC MESENTEROIDES*

Mani Sharma and APS Mann

Department of Biotechnology, Hans Raj Mahila Maha Vidyalaya, Jalandhar, Punjab, India

ABSTRACT

The present studies were carried out to investigate the post harvest deterioration in sugarcane juice due to presence of *Leuconostoc mesenteroides* bacteria. The bacteria released sucrose hydrolyzing enzymes in the juice viz. invertase and dextransucrase, which hydrolyzed sucrose into glucose and fructose, thus reducing the sucrose content in the juice. *Leuconostoc mesenteroides* bacteria was isolated from slime obtained from two sugar mills at Budhewal (Broth I) and Morinda (Broth II). Purified strain no. 867 procured from institute of Microbial technology (IMTECH), Chandigarh was used as standard check. Sucrose hydrolyzing enzymes i.e. dextransucrase was isolated from L. mesenteroides. In addition to catalyzing the sucrose hydrolysis, dextransucrase also synthesized dextran due to its transferase activity. Maximum activity of the enzymes was associated with cell walls of the *Leuconostoc mesenteroides isolated from Broth I, Broth II and standard 867.* Effect of temperature was also studied on production of dextran by the bacteria islated from broth I and broth II.The present studies have potential applications in sugar industry for minimizing dextran problem and point to the need of estimating dextran levels and bacterial count, in sugarcane juice as a part of quality control for improving sugar recovery and sugar quality.

INTRODUCTION

The post harvest deterioration of sugarcane is one of the most vexing problems of sugar industry and has attracted widespread attention in recent years. Sugar cane is sensitive to enormous sucrose losses induced by physio-chemical and microbial changes, the severity being increased during the time lag between harvest and crushing in the mills. A study revealed around 13.0 kg sugar loss/ton cane milled due to harvest to milling delays (Solomon et al 2007).

Sugar technologists have reported a variety of cane deterioration products to confirm cane deterioration and delay (cut-to-crush time), which have been used to predict and control process problems at the factory. Such deterioration products have included high invert concentration, microbial (yeast, bacteria and fungi) contamination and polysaccharides (dextrose). (Eggleston G, 2002).

The delay in transport of sugarcane from field to sugar mill increases the infection by *Leuconostoc mesenteroides* thereby faster deterioration of cane quality. The polysaccharide dextran directly and negatively affects the efficiency of factory processing as they interfere with the crystallization and pull down sugar recovery (Singh et al, 2008).

Remaud et al (1999) observed that dextransucrase from *Leuconostoc mesenteroides* could be used to produce a diversity of controlled oligosaccharides. So, in the present investigation, the post harvest deterioration of sugarcane by *Leuconostoc mesenteroides* due to production of dextran in the sugarcane juice under the effect of temperature and environment conditions was studied.

MATERIALS AND METHODS

The experiment was conducted at the Deptt. of Biochemistry in Punjab Agricultural University, Ludhiana. The slime which was deposited in crushers of Budhewal and Morinda Sugar Mills, during extraction of sugarcane juice was collected and stored at 4°C. For isolation of *Leuconostoc mesenteroides* colonies, enriched nutrient agar medium was used, which was enriched with Brain heart infusion broth.

By Comparing the characters of each different colony with pure culture of L.*mesenteroides* (strain no. 867) procured from IMTECH, Chandigarh, the colonies showing similar morphology were selected and proceeded further. Enriched nutrient broth having bacterial colonies was centrifuged at 8000g for 20min.

For assay of wall bound and extracellular dextransucrase, enzyme extract was prepared from cell debris and supernatant respectively by the method of Batta et al (1991). Assay of dextransucrase was carried out by the method of Kobayashi and Matsuda (1974), while the amount of dextran in juice was estimated by method of Roberts (1983). Proteins in the bacterial cell debris and supernatant were estimated by the method of Lowry et al (1957).

RESULTS AND DISCUSSION

Dextransucrase (E.C.2.4.1.5) is an extracellular glycosyltransferase, which catalyses the transfer of D-glucopyranosyl residues from sucrose to dextran, while fructose is released. Its synthesis in wild type strains of L. *mesenteroides* is actually induced by growth on sucrose (Naessens et al, 2005).

The present study shows that, L. *mesenteroid*es colonies isolated from Budhewal sugar mill slime sample (Broth-I) and Morinda sugar Mill slime sample (Broth-II) produced dextransucrase in extracellular tissue (supernatant), intracellular tissue (cell extract) and wall bound (cell debris).The higher transferase activity of dextransucrase was associated with cell wall of the bacterium. In Budhewal sample (Broth-I), the activity was 1.59mg dextran/hr/mg pro., 1.89mg dextran/hr/mg pro. and 2.64mg dextran/hr/mg/pro. for extracellular, intracellular and wall bound tissue resp. Similar results were reported in the Morinda Mill (Broth-II) sample(table 1). When sucrase activity was studied, it was observed that intracellular activity of enzyme was more than that of extracellular and wall bound enzyme activities for both, Broth-I and Broth-II (table 2). So the sucrase activity of dextransucrase is associated with the cells of *Leuconostoc mesenteroides.*

Similar results were reported by Singh et al (2008), extracellular dextransucrases secreted by Leuconortoc bacteria increased sharply at the cut ends of harvested cane, which converts its sucrose into dextran. This enzyme secreted mostly by Leuconostoc bacteria, not only catalyses dextran synthesis from sucrose to form oligosaccharides like leucrose and palatinose, is also a potential criteria for cane deterioration (Eggleston and Legendre, 2003).

Cortezi et al (2005), reported that dextransucrase production by *Leuconostoc mesenteroides*_was affected by temperature and sucrose concentration . In this study, estimation of dextran content from the sugarcane juice was also done to correlate the increase in bacterial(L. *mesenteroides*) population with the dextran content of juice in winter(15-20^{o}c) as well as summer(30 – 40^{o}c) from Budhewal & Morinda sugar mills. It was observed that as the optimum temperature for L. *mesenteroides* is 30 – 40^{o}c , so growth and multiplication is higher in early summer season, so dextran content was more in sugarcane juice during summer i.e. 8.33 mg dextrans / 5.0 ml juice in Broth I and 7.52 my dextrans / 5.0 ml juice as compared to during winter i.e. 6.94 and 5.42 mg dextrans / 5.0 ml juice from Broth II(table 3,4). Cortezi etal(2005) reported similar results, that optimum temp for enzyme dextransucrase from L. *mesenteroides* strain NRRL B512 was 25^{o}c.

From the above study it may be concluded that the environmental and storage conditions affect the sucrose content of the sugarcane juice and increase the dextrans, however dextrans are only the partial contributors.

REFERENCES

1. Batta S K, Singh J, Sharma K P and Singh R (1991) Kinetic properties and inhibition of soluble acid invertase from sugarcane juice. *Plant Physiol*. *Biochem* **29**: 418-19
2. Cortezi M, Monti R and contiero J (2005) Temperature effect on dextransucrose production by *leuconostoc Merenteroides* FT 045 B isolated from alchoal and sugar mill plant. *African J Biotech* **4(3)**: 279-85
3. Eggleston G (2002). Deterioration of cane juice- sources and indicators. *Food chem* **78**: 95-103
4. Kobayashi M and Matsuda K (1977). Structural characterization of dextran synthesized by dextransucrase from *leuconstoc mesenteroides* NRRL – 1299. *Agric Biol Chem* **41**: 1931-37
5. Lowry O H, Rosebrough N J, Farr A L and Randall R J (1951) Protein measurement with the Folin Phenol Rogent. *J Biol Chem* **193**: 265-77
6. Naessens m, Cerdobbel A, Soetaert W and Vandamme E J (2005). Leuconostoc dextransucrase and dextran: production, properties and applications. *J Chem tech. Biotech* **80(8):** 845-60
7. Remaud Simeon M, Quirasco M, Mousan P and Lopez Munguia A (1991) Experimental behavior of a whole cell immobilized dextransucrase biocatalyst in batch and packed bed reactors. *Bioprocess Engineering* **20 (4)** :289-95
8. Roberts E J (1983) A quantitative method for the determination of fructose in blood and urine. *J Biol Chem* **107**: 15-22
9. Solman S, Shrivastava A K and Yadav RL (2007). Stratergies to minimize post harvest sucrose lossesin sugarcane: an overview. Proc. 68th Annual convention STAI, 22-24th August, 2007. PP: 112-21
10. Singh P, Solomon S, Shrivastava A K, Prajapati C P and Singh R K (2008). Post harvest deterioration of sugarcane and its relationship with the activities of invertase and dextransucrose during late crushing season in subtropics. *Sugar tech* **10(2)**: 133-36

Table 1: Comparison of Transferase Activity of dextran sucrase enzyme from Leuconostoc mesenteroides isolated from Broth I, Broth II & Standard strain 867

Source	Transferase activity (mg dextran / hr/mg protein)		
	supernatant broth (extracellular)	Cell extract (Intracellular)	Cell debris (wall bound)
broth 1	1.59	1.89	2.64
broth 11	1.86	2.61	3.17
standard strain 867	1.19	1.51	2.45

Table 2: Comparison of Sucrase Activity of dextran sucrase enzyme from Leuconostoc mesenteroides isolated from Broth I, Broth II & Standard strain 867

Source	sucrase activity (nmol RS / hr/mg protein)		
	Supernatant broth (extracellular)	Cell extract (Intracellular)	Cell debris (wall bound)
broth 1 (Budhewal)	3592	9394	7725
Broth 1 (Morinda)	6812	15443	7121
standard strain 867	4762	10185	6491

Table 3: Dextran estimation from sugarcane juice (Co. 89003) from Budhewal and Morinda suger mill during winter season(10 – 15^{o}c)

Days after harvesting (DAH)	Dextran (mg dextran / 5.0 ml juice)	
	Budhewal	Morinda
0	0.39	0.32
3	1.49	1.11
7	3.02	2.93
10	5.03	4.68
15	6.94	5.42

Table 4: Dextran estimation from sugarcane juice (Co. 89003) from Budhewal and Morinda suger mill during summer season(30 – 40^{o}c)

Days after harvesting (DAH)	Dextran (mg dextran / 5.0 ml juice)	
	Budhewal	Morinda
0	0.36	0.32
3	2.19	1.93
7	3.42	3.31
10	5.78	5.6
15	8.33	7.52