PRODUCTION AND CHARACTERIZATIONOF LEVAN BY BACTERIA FOUND IN SOLID WASTES

Chetan D.M[1]*,Dr. Narendra Kumar S[2], Raghavendra H.L[3]and Venkatesh Kamath H[4]and Anil Kumar H.S[5]

[1]Department of Biotechnology, NMAM Institute of Technology-Affiliated to NITTE (Deemed to be University), Nitte, Karnataka- 574110, India

[2]Assistant Professor, Department of Biotechnology R. V College of Engineering, Bangalore

[3]Center for Biotechnology,Pravara Institute of Medical Sciences (Deemed to be University),Loni-413736, Rahata Taluk, Ahmednagar District, Maharashtra, India

[4,5]Department of Biotechnology, NMAM Institute of Technology-Affiliated to NITTE (Deemed to be University), Nitte, Karnataka- 574110, India

ABSTRACT

Levans are organic fructose polymers that can be found in a variety of plants and microbial products. Levan was produced using bacteria found in solid waste in an attempt to do so. The separation of organisms from solid waste. The isolated organisms were identified to be Bacillus pasturii, by using variousbiochemical tests. This organism screened for Levan production. Levan production tested toproduce by Shake flask studies.It dissolves easily in water. Levan has not been used, but if it were, it might be valuable in the food industry and for other industrial uses,it seen that 5ml of Bacillus pasteurii (orange) have the more concentration than the Bacillus pasteurii (peas). we also observed that as the amount of sample increases, the concentration of fructose also increases.it can be seen that 5ml of Bacillus pasteurii (orange) have concentration of fructose double than the Bacillus pasteurii (peas). We got high concentration of fructose in Bacillus pasteurii (peas) than the Bacillus pasteurii (orange).it can be seen that the high concentration of fructose present in the Bacillus pasteurii (orange) than the Bacillus pasteurii (peas).

***Keywords**: Levan, 6-kestose, and Shake flask studies*

DOI Number: 10.14704/NQ.2022.20.12.NQ773701 **NeuroQuantology2022;20(12): 3744-3752**

1. INTRODUCTION

1.1Solid Waste

Unwanted material that cannot naturally flow into streams or escape into the atmosphere is disposed of as solid waste by humans. These solid, non-liquid, non-gaseous wastes are a byproduct of numerous human activity. These lead to pollution of the soil, water, and air. (1993; Misra and Mani). [13] Meeting human needs for food, drink, air, space, shelter, and movement inevitably results in waste. Product recovery or recycling can drastically alter the quantity and quality of waste in any materials processing, yet all operations eventually produce some trash. (1992; Swarup et al.). Although the outbreak of SW is not a recent phenomena, it now poses the risk of of uses. [3]. becoming the "third pollution" after air pollution and water pollution due to the advancement of industry and population boom.[12]

1.2 LEVAN

Fructose, a sugar present in a variety of plant and microbial compounds, is naturally polymerized to form levan [2]. This review paper introduces Levan, an intriguing -(2,6)-linked fructose polymer with a peculiar set of characteristics. Levan is naturally produced from sucrose by a variety of microorganisms and some plant species [14]. Bacterial levans frequently have molecular weights greater than 500,000 Da and are frequently branched, forming compact nanospheres with a variety

eISSN 1303-5150

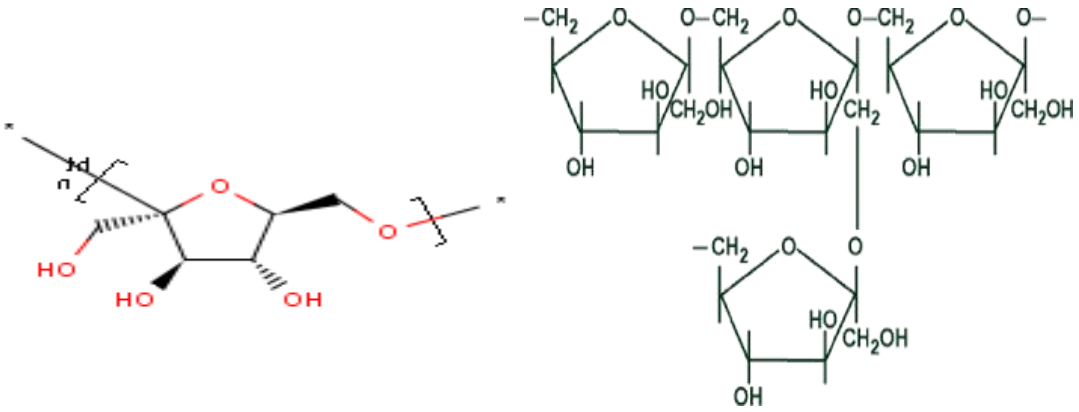

Fig a: Levan Structure shows the Fructan Backbone and the Method for Attachment of Occasional Branches levan occasional branches.

3. METHODOLOGY

3.9.1 Preparation of microbial culture [8]

1. The reactivated culture of bacillus pasteurii was transferred aseptically to nutrient broth. Incubated for 48hours at 30°C.Purity was observed after 48 hours.Cultures were then transferred to 250 mL Erlenmeyer flask, containing 150 mL Nutrient Broth and placed on a rotary shaker for 24 hours at 200 rpm at 30°C.This was repeated in 5 **[11]**
2. **Polymer production:** Prepared culture was transferred to 5 culture bottles. Each culture bottles was injected with 1%, 2%, 3%, 4%, 5%, 6% (v/v) of homogeneous mixture from the flask bacterial culture each time. Kept in incubator shaker at 30°C with constant aeration 200 rpm. Kept for 5 days in incubator shaker. Samples were collected after 5 days.**[8]**
3. **Polymer measurement:**After growth, the culture was centrifuged at 2400 rpm for 10 min to remove bacterial cells and dialyzed to remove unfermented sugars and low molecular weight fermentation products. Levan was precipitated by adding 3 volumes of methanol to the cell-free supernatant. The mixture was then vortexed for 10-15 seconds and then centrifuged at 2400 rpm for 10 minutes before isolating the precipitated polysaccharide. The pH was adjusted to 2.0 and the samples were heated in boiling water. After cooling, the samples were analyzed by UV spectroscopy[**10]**

Fig 1: Bacterial Culture

eISSN 1303-5150

Fig 2: Inoculum for Batch Culture

Fig 3: Polymer Measurement of Peas and Orange Sample

3.9.4 UV- Visible Spectroscopy Method [9]

1. Approximately 1 mL of pre-chilled Levan solution was taken and quickly mixed with 4 mL of concentrated hydrochloric acid in a test tube. Vortex for 2 minutes in an ice bath. After adding HCl directly to the liquid surface, the temperature of the mixture rises rapidly within 10-15 seconds. The reaction was then heated to 70° C. in a water bath for 2 minutes. The mixture was then cooled in an ice bath for 2 minutes. Finally, the tube was placed in a 25°C water bath for 5-10 minutes. Absorbance was read at 340 nm. Production and extraction of levan by Staphylococcus aureus: S. aureus was used to create Levan. Organisms grew on the medium. After several days of growth at 30° C. on a rotary shaker, the medium was centrifuged to remove bacterial cells. Levan was then precipitated with 1.5 volumes of ethanol. The precipitate was lyophilized for 1 day. A preliminary test was performed. Using the absorbance obtained at 340 nm and the standard plot, the concentration of levan produced from organisms is: The amount of levan extracted from the fermentation of Bacillus pasteurii was detected in the supernatant and filtered.

• Levan was precipitated by adding 3 mL of methanol to the cell-free supernatant. The mixture was then vortexed for 10 seconds and then centrifuged at 24,000 rpm for 10 minutes before isolating the precipitated polysaccharide. • The pH was adjusted to 2 and the samples were heated in boiling water.

• After cooling, the samples were analyzed with a UV spectrophotometer.

Fig 4a: Bacterial Culture **Fig 4b: Analysis Sample**

eISSN 1303-5150

ANALYSIS OF SAMPLE

Table 4.1: 1% inoculum obtained from orange and peas inoculated in nutrient broth

Sl. NO	SAMPLE (ml)	D/W (ml)	2 N HCL (ml)	Boiling Water Bath For 5 min	Neutralise the Sample	Boiling Water Bath for 5 min	2 N HCL (ml)	Vortex and ice bath for 5min	Water bath for 10min at70°C	Ice bath for 5min	OD at 340	CONC (mg/ml)
1	Blank	1	-				4				0	0
2	1(Peas)	-	2				4				0.248	0.12
3	2	-	2				4				0.252	0.12
4	3	-	2				4				0.304	0.147
5	4	-	2				4				0.39	0.189
6	5	-	2				4				0.422	0.2
7	6(Orange)	-	2				4				0.29	0.141
8	7	-	2				4				0.33	0.16
9	8	-	2				4				0.374	0.18
10	9	-	2				4				0.523	0.254
11	10	-	2				4				0.515	0.25

Table 4.2 : 2% inoculum obtained from orange and peas inoculated in nutrient broth.

Sl. NO	SAMPLE (ml)	D/W (ml)	2 N HCL (ml)	Boiling water bath for 5 min	Neutralise the Sample	Boiling Water Bath for 5 min	2 N HCL (ml)	Vortex and ice bath for 5min	Water bath for 10min at70°C	Ice bath for 5min	OD at 340	CONC (mg/ml)
1	Blank	1	-				4				0	0
2	1(Peas)	-	2				4				0.828	0.4026
3	2	-	2				4				0.516	0.2509
4	3	-	2				4				0	0
5	4	-	2				4				0.819	0.398
6	5	-	2				4				0.825	0.4
7	6(Orange)	-	2				4				1.032	0.501
8	7	-	2				4				0.743	0.361
9	8	-	2				4				1.069	0.519
10	9	-	2				4				0.698	0.339
11	10	-	2				4				0.204	0.099

Table 4.3: 3% inoculum obtained from peas and orange inoculated in nutrient broth

Sl. NO	SAMPLE (ml)	D/W (ml)	2 N HCL (ml)	Boiling Water Bath For 5 min	Neutralise the Sample	Boiling Water Bath for 5 min	2 N HCL (ml)	Vortex and ice bath for 5min	Water bath for 10min at70°C	Ice bath for 5min	OD at 340	CONC (mg/ml)
1	Blank	1	-				4				0	0
2	1(Peas)	-	2				4				0.490	0.238
3	2	-	2				4				0.79	0.359
4	3	-	2				4				0.880	0.427
5	4	-	2				4				0.735	0.357
6	5	-	2				4				0.594	0.288
7	6(Orange)	-	2				4				0.961	0.467

eISSN 1303-5150

8	7	-	2				4				0.610	0.496
9	8	-	2				4				0.305	0.148
10	9	-	2				4				0.549	0.668
11	10	-	2				4				0.479	0.232

Table 4.4: 4% inoculum obtained from peas and orange inoculated in nutrient broth

SI. NO	SAMPLE (ml)	D/W (ml)	2 N HCL (ml)	Boiling Water Bath For 5 min	Neutralise the Sample	Boiling Water Bath for 5 min	2 N HCL (ml)	Vortex and ice bath for 5min	Water bath for 10min at70°C	Ice bath for 5min	OD at 340	CONC (mg/ml)
1	Blank	1	-				4				0	0
2	1(Peas)	-	2				4				0.466	0.226
3	2	-	2				4				0.460	0.2235
4	3	-	2				4				0.650	0.3159
5	4	-	2				4				0.617	0.299
6	5	-	2				4				0.346	0.1681
7	6(Orange)	-	2				4				0.508	0.2468
8	7	-	2				4				0.518	0.251
9	8	-	2				4				0.594	0.288
10	9	-	2				4				0.583	0.283
11	10	-	2				4				0.285	0.138

Table 4.5: 5% inoculum obtained from peas and orange inoculated in nutrient broth

SI. NO	SAMPLE (ml)	D/W (ml)	2 N HCL (ml)	Boiling water bath for 5 min	Neutralise the Sample	Boiling Water Bath for 5 min	2 N HCL (ml)	Vortex and ice bath for 5min	Water bath for 10min at70°C	Ice bath for 5min	OD at 340	CONC (mg/ml)
1	Blank	1	-				4				0	0
2	1(Peas)	-	2				4				0.358	0.1739
3	2	-	2				4				0.480	0.233
4	3	-	2				4				0.807	0.392
5	4	-	2				4				0.757	0.367
6	5	-	2				4				0.651	0.316
7	6(Orange)	-	2				4				0.750	0.3645
8	7	-	2				4				0.543	0.2638
9	8	-	2				4				0.875	0.425
10	9	-	2				4				1.206	0.586
11	10	-	2				4				1.766	0.858

Table 4.6: Standard Calibration

SI. NO	CONC (mg/ml)	D/W (ml)	2 N HCL (ml)	Vortex and ice bath for 5min	Water bath for 10min at70°C	Ice bath for 5min	OD at 340
1	0	1	4				0
2	0.2	0.8	4				0.907

eISSN 1303-5150

3	0.4	0.6	4				1.151
4	0.6	0.4	4				0.832
5	0.8	0.2	4				0.575
6	1.0	0	4				0.727

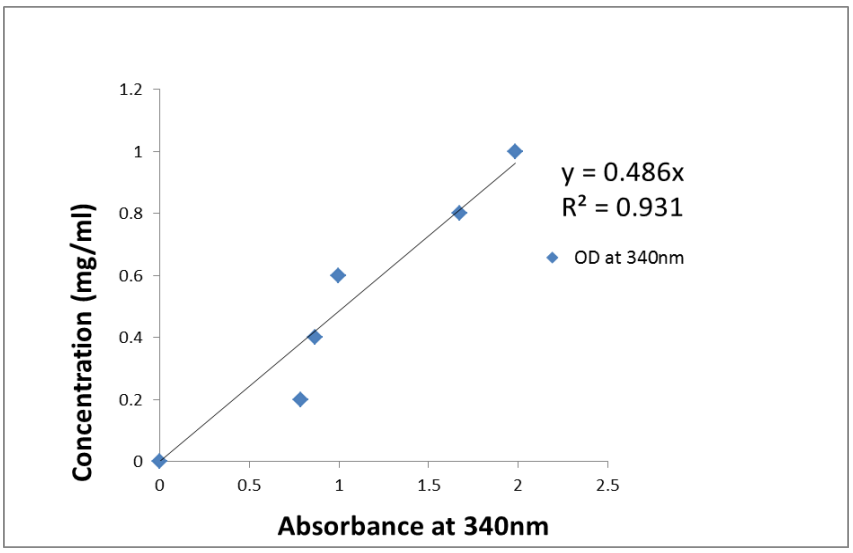

Fig 12: Standard plot for estimation of fructose

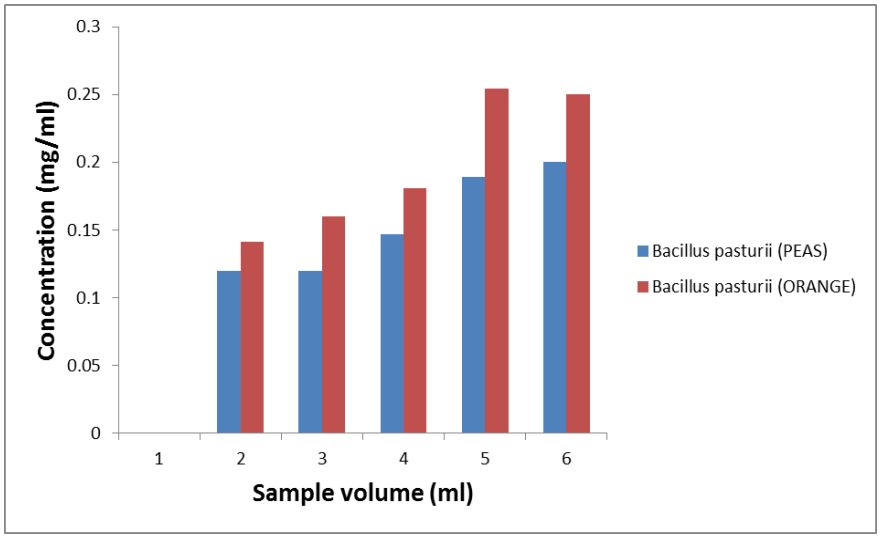

Fig 12.1: 1% inoculum obtained from peas and orange inoculated in nutrient broth

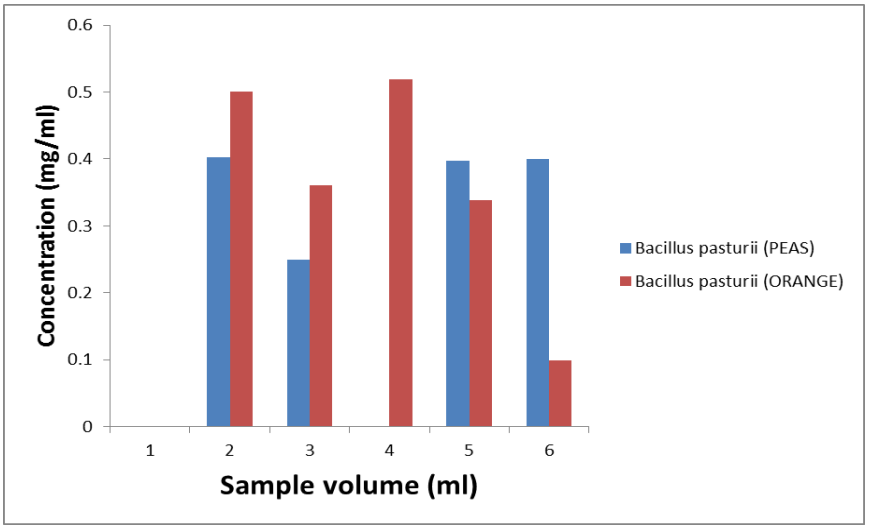

Fig 12.2: 2% inoculum obtained from peas and orange inoculated in nutrient broth

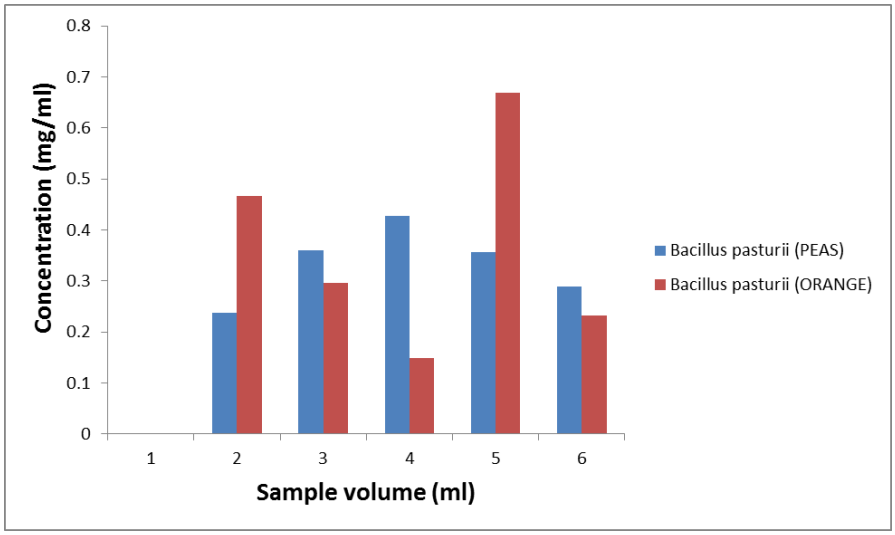

Fig 12.3: 3% inoculum obtained from peas and orange inoculated in nutrient broth

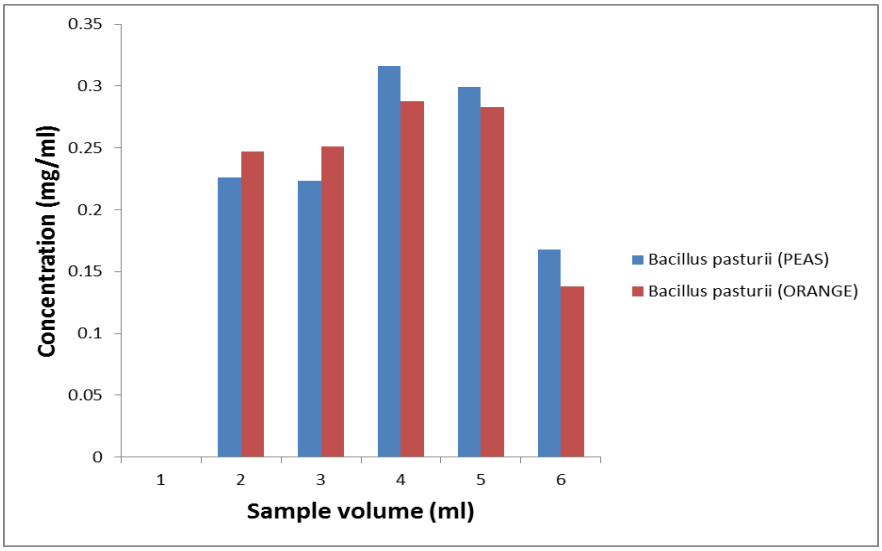

Fig 12.4: 4% inoculum obtained from peas and orange inoculated in nutrient broth

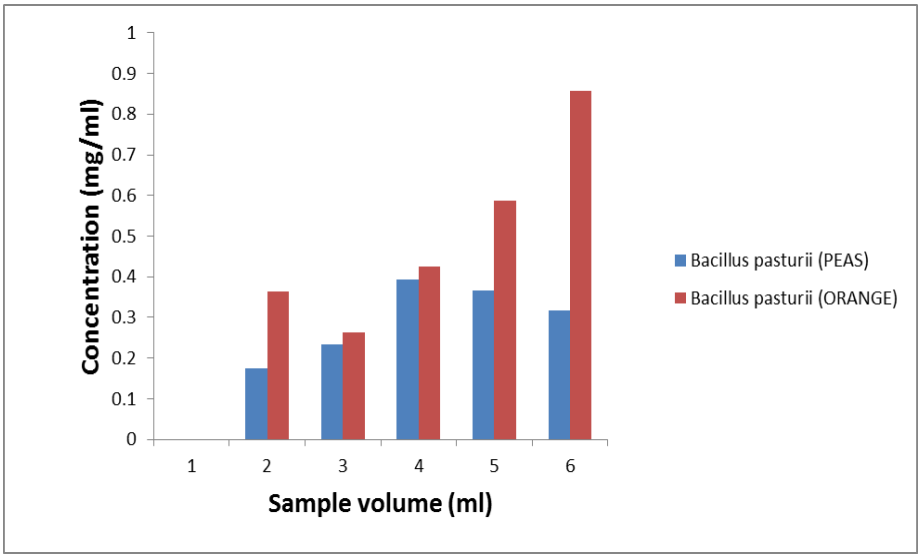

Fig 12.5: 5% inoculum obtained from peas and orange inoculated in nutrient broth

eISSN 1303-5150

RESULTS AND DISCUSSIONS

Bacillus pasteurii was isolated from solid waste and used for production of Levan.

• From the graph 1, it seen that 5ml of *Bacillus pasteurii* (orange) have the more concentration than the *Bacillus pasteurii* (peas). In this graph we are observe that as the amount of sample increases, the concentration of fructose also increases. So we got higher concentration in 1% graph.• From the graph 2, it is represented as a hypozoid graph, it was seen that 4ml of *Bacillus pasteurii* (orange) have more concentration of fructose than the *Bacillus pasteurii* (peas). But 6ml of *Bacillus pasteurii* (peas) contains concentration of fructose thrice than the *Bacillus pasteurii* (orange).• From the graph 3, it can be seen that 5ml of *Bacillus pasteurii* (orange) have concentration of fructose double than the *Bacillus pasteurii* (peas).In this graph we observe that the 3,4 and 6ml of *Bacillus pasteurii* (peas) leads than the *Bacillus pasteurii* (orange).• From the graph 4, we got high concentration of fructose in *Bacillus pasteurii* (peas) than the *Bacillus pasteurii* (orange).• From the graph 5, it can be seen that the high concentration of fructose present in the *Bacillus pasteurii* (orange) than the *Bacillus pasteurii* (peas). In this graph, we observe that the concentration of fructose in the *Bacillus pasteurii* (peas) increases 2 to 4ml and it suddenly drop down to 4 to 6ml. So, 5% of inoculum have more concentration of Levan compared to 1%, 2%, 3% and 4% inoculums.

CONCLUSION

Our current results indicate that microbial fermentation organisms found in solid waste can be used for levan production. The levan thus obtained is inexpensive and can be used in medical, food, pharmaceutical, cosmetic and commercial industrial sectors after further purification and standardization. Systematic studies have been conducted to extract levan by bacteria isolated from solid waste, and low concentrations of levan can be obtained from the isolated bacteria. Room for improvement is to develop methods to increase yield by changing media and using other microorganisms. This connection is secure and our research enables the feasibility of new industrial applications.

REFERENCES

1. Garg, S. (2006): A project report on the assessment of solid waste management of solid waste management through public participation in the valley of flowers national park.
2. Han YW. (1990): Microbial Levan: United States of Department of Agriculture, agricultural Research Service, New Orleans. 35: 171-94.
3. Oner ET, Hernandez L, Combie J (2016): Review of Levan Polysaccharide: From a Century of Past Experiences to Future Prospects. Sep-Oct; 34(5): 827-844.
4. J. Combie (2006) : Properties of Levan and Potential Medical Uses; Montana Polysaccharides Corporation, 1910-107 Lavington, Rock Hill, SC29732; Polysaccharides for Drug Delivery and Pharmaceutical Applications pp263-269.
5. Soon Ah Kang, Ki-Hyo Jang, Jeong-Woo Seo, Ki Ho Kim, Young Heui Kim, Dina Rairakhwada, Mi Young Seo, Jae Ok Lee, Sang Do Ha, Chul-Ho Kim and Sang-Ki Rhee (2009): Levan: Applications and Perspectives. Microbial Production of Biopolymers and Polymer precursors: Applications and Perspectives (Edited by: Bernd H. A. Rehm). Caister Academic Press, U.K. .
6. Srikanth R, Reddy CH, Siddartha G, Ramaiah MJ, Uppuluri KB (2014) : Review on Production, Characterization and Applications of Microbial Levan. 12.003
7. Youn W. Han and Margaret A. Clarke (1990): Production and Characterization of Microbial Levan. 38, 393-396.
8. Ghaly, A.E., F. Arab, N.S. Mahmoud and J. Higgins (2007): Department of Process Engineering and Applied Sciences

eISSN 1303-5150

Dalhousie University, Halifax, Nova Scotia, Canada B2J2X4 : Production of levan by Bacillus licheniformis for Use as a Soil Sealant in Earthen Manure Storage Structures. 3 (2): 47-54.

9. Mamay, Deana Wahyuningrum, RukmanHertadi (2015): Isolation and Characterization of Levan from Moderate Halophilic Bacteria Bacillus Licheniformis BKAG21. 292-298.
10. Youn W, Han; Margaret A. Clarke (1996): Production of fructan (levan) polyfructose polymers using bacillus polymyxa.
11. Tian F, Inthanavong L, Karboune S (2011): Purification and characterization of levansucrases from Bacillus amyloliquefaciens in intra- and extracellular from useful for the synthesis of levan and fructooligosaccharides.
12. Jaco Franken, Brandt, Siew L. Tai, Florian F. Bauer (2013): Biosynthesis of Levan, a Bacterial Extracellular Polysaccharide, in the Yeast Saccharomyces Cerevisiae.
13. Safaa AL-Deen Ahmed Shanter AL-Qaysi, Zaid Akram Thabit, Sanaa Basheer Kadhem (2016): Biosynthesis of Levan, a Bacterial Extracellular Polysaccharide, in the Yeast Saccharomyces Cerevisiae. 83-96.
14. V. K. Ananthalakshmy and P. Gunasekaran (1999): Optimization of levan production by Zymomonasmobilis. 1678-4324.