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Biosynthesis and Characterization of Dextran Produced by *Staphylococcus Species*

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Abstract:

Initially, Buffalo and Cow curd samples were used for isolation of bacterial samples for Dextransucrase production. The modified MRS agar medium containing Peptone, beef extract, yeast extract, glucose, Sodium acetate, poly-sorbate 80, di-potassium hydrogen phosphate, tri-ammonium citrate, Magnesium sulphate, Manganese sulphate, was used for isolation of suitable enzyme used for production of dextransucrase with 2% Sucrose as a main substrate. Then, Isolate with higher dextransucrase activity was selected using DNS Assay (Dinitrosalicylic acid method). After that the isolate was identified up to the genus level using ABIS online laboratory tool by morphological and biochemical characterization for example, Grams staining, Indole test, Methyl red test, etc. Moreover, after selection of suitable isolate certain parameters were observed for maximum production of dextransucrase enzyme. Finally, for production of dextran, dextransucrase was produced by incubation of 24hours old seed medium into selected production medium at optimum culture conditions. The Supernatent obtained by centrifugation containing dextransucrase was used for production of dextran in 2% Sucrose Solution.

Introduction:

Dextansucrase is an industrially important enzyme for the production of dextran by catalyzing the transfer of glucosyl residues from sucrose. The dextran produced from sucrose by dextransucrase has many important applications like As-anticoagulant agent, in anticancer therapy, in antibacterial and antiviral drugs, in cosmetics etc. It is basically a gulosyltransferase enzyme that synthesis dextran from sucrose by catalyzing the transfer of glucosyl residue from sucrose to the growing glucan polymer and eliminating fructose as a byproduct. Sucrose is the only known substrate able of inducing this enzyme production (Santos et al., 2000). Different researchers have reported that dextransucrase exists in two different forms, that are single or multiple forms having the molecular weights in the range from 64,000 to 245,000 (Kobayashi and Matsuda 1980, 1986; Miller et al., 1986; Willemot et al., 1988; Fu and Robyt 1990; Goyal and Katiyar, 1994). Dextransucrases are closely related to the other enzymes like amylosucrase, cyclo-dextrin glucanotransferase, amylomaltase, and a-amylase from families GH13 and GH77 (Cantarel et al., 2008). It is a polysaccharide consisting of glucose monomers linked mainly (95%) by α (1–6) bonds. Synthesizes of dextran from sucrose involves the covalent dextranosyl and glucosyl enzyme intermediates, in addition to sucrose, if other carbohydrates (such as maltose, iso-maltose, etc.) are present, some of the glucosyl groups are transferred to the these

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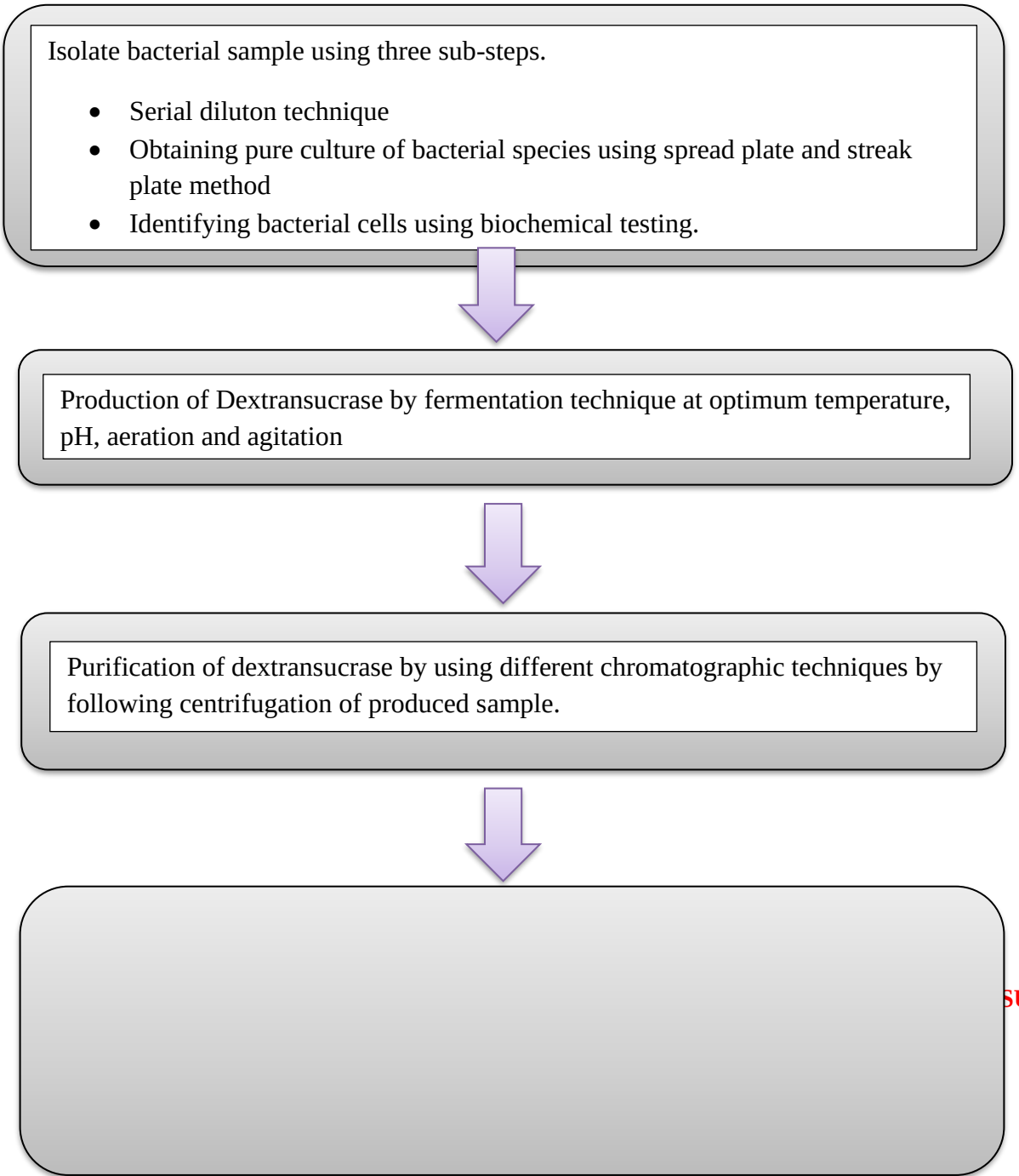
carbohydrates (“acceptor reaction”) and are diverted from synthesising dextran to form acceptor products. The three reactions catalysed by Dextransucrases are as follows: Polymerisation of glucose moiety of sucrose, Glucose transfer to acceptors, Sucrose hydrolysis (Plou et al., 2002).There are many factors which affect the activity of dextransucrase for production of detran such as temperature, pH, sucrose concentration, aeration and agitation. These optimum culture conditions are necessary to maintain for high yield production of dextransucrase. There are many different methods exist for purification of dextran such as precipitation by salt and solvent, fractionation with polyethylene glycol, chromatographic techniques and phase partitioning etc. The production of dextransucrase and its characterization can be explained in simple steps as drown in figure 1.



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- Production of dextran using dextransucrase and solution containing 2% Sucrose as substrate
- Purification of produced dextran using ethanol for its precipitation or by Fractionation with polyethylene glycol
- Characterization of dextran by solubility testing in different solvents and by optimizing viscosity (η) using Viscometer.

Figure 1: The production of dextransucrase and its characterization (Source: Primary)

Material and Method:

1. Sample Collection: Curd Samples were collected from nearby areas of Ambala City (India). The details are given in table 1.1

S.NO.	TYPE OF CURD SAMPLE	LOCATION OF SAMPLE
1	Buffalo Curd	V.P.O Panjokhra Sahib, Ambala, Haryana (India)
2	Cow Curd	V.P.O Panjokhra Sahib, Ambala, Haryana (India)
3	Verka Curd	Derabassi, Punjab (India)
4	Local Dairy Curd	Satkar Dairy, Ambala, Haryana (India)

Table 1.1: Different curd samples taken from different regions. (Source: primary)

2. Isolation of dextransucrase producing microbes: The culture (sample) was isolated from curd by serial dilution technique. The sample from 10^{-1} to 10^{-6} dilutions was taken with the help of micropipette and then spread plated on MRS agar medium. The micro-aerophilic condition was created by tightly packing the plates using Para film and then incubation was done at 25°C for 36 hours. The plates were observed for characteristic colonies and then streaking of single colonies was done to obtain pure culture.

3. Selection of Isolate: The dextransucrase positive microorganisms were isolated on the basis of colony characteristics on MRS agar plates were further screened for dextransucrase activity. The strains were grown in MRS broth having same composition of MRS medium except agar and incubated at 37^{0} C for 48 hours in temperature controlled incubator shaker at 150rpm. The

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culture broth was then centrifuged at 10,000 g for 10 minutes. Hence, dextransucrase activity was determined by DNS Assay (Dinitrisalicylic acid).

4. Identification of bacterial Culture: Isolate with higher dextransucrase acitivity was selected and further characterized using Bergey's manual of Determinative Bacteriology. By morphological and biochemical characterization viz.; Gram staining, indole test, catalase test, oxidase test, nitrate test, urease test etc., the isolate was identified at genus level using ABIS online laboratory tool.

5. Medium Selection and Optimization of various culture conditions:

5.1 Selection of medium for maximum dextransucrase production by Staphylococcus species: Medium producing high amount of dextransucrase activity in fermentation broth was needed to be selected and optimized. 5 different media's were taken for optimization for high dextransucrase activity (table 1.2). Seed was prepared in MRS broth and then 5ml was inoculated into each medium and then incubated for 24 h at 37^0 C (150 rpm speed). Dextransucrase activity was evaluated for each medium.

5.2 Optimization of Sucrose Concentration for maximum dextransucrase production by Staphylococcus species: To find out optimum sucrose concentration for maximum dextransucrase activity, M-3 media with different sucrose concentration (2%, 4%, 6%, 8%, 10%) were prepared and inoculated with 24 h old seed medium (5%,v/v) containing (5%,w/v) sucrose. Flasks were incubated at 370C for 24 hours (150rpm). Dextransucrase activity was tested by DNSA method.

Medium No.	Composition	References
M-1	Sucrose (2%), Yeast extract (0.5%), K_2HPO_4 (2.0%), $MgSO_4$ ($7H_2O$) 0.02%, $MnSO_4$ ($4H_2O$) 0.001%, $FeSO_4$($7H_2O$) 0.001%, $CaCl_2$ (0.001%), NaCl(0.001%).	Bhavani et al., 2014
M-2	Sucrose (10%), Yeast extract (0.5%), Peptone (0.5%), K_2HPO_4 (1.5%), NaCl (0.001%), $MgSO_4.7H_2O$ (0.001%), $MnCl_2.H_2O$ (0.001%), $CaCl_2$ (0.01%).	Al-doori et al., 2015

M-3	Sucrose (5%), Tween 80(0.1%), Yeast extract (1.5%), Peptone (2.0%), K_2HPO_4 (1.5%).	Ghosh et al., 2018
M-4	Sucrose (5%), Yeast extract (2.0%), K_2HPO_4 (2.0%), $MgSO_4.7H_2O$ (0.02%), $MnSO_4.2H_2O$ (0.001%), NaCl (0.001%), $CaCl_2.2H_2O$ (0.001%), $FeSO_4.7H_2O$ (0.001%).	Shukla et al., 2010
M-5	Sucrose (150%), Bactopeptone (5.0%), Yeast extract (5.0%), K_2HPO_4 (15%), $MnCl_2.H_2O$ (0.01%), NaCl (0.01%), $CaCl_2$ (0.05%).	Sarwat et al., 2008

Table 1.2 Different Media used for production of dextransucrase. (Source: Primary)

5.3 Optimization of medium pH for maximum dextransucrase production by Staphylococcus species: For optimization of optimum pH for maximum dextransucrase production M-3 Media with different pH (5.0-9.0) were prepared and inoculated with 24 h old seed (5%,v/v) Containing (2%,w/v). Flasks were incubated at 37^0C for 24 h (150rpm). Dextransucrase activity was assayed in the supernatant. The final pH of the fermentation broth was noted.

5.4 Optimization of Inoculum size for maximum dextransucrase production by Staphylococcus species: For optimization of inoculum size, M-3 medium was prepared in 5 flasks containing 2% Sucrose and pH 6 was adjusted for each medium flask then inoculated with 24 h old seed culture with different inoculum concentration (4%, 8%, 12%, 16%, 20%). The inoculated production medium was incubated at 37^0C for 24 h (150rpm) in an incubator shaker. Maximum dextransucrase production was determined in each set of experiments.

5.5 Optimization of Nitrogen source for maximum dextransucrase production by Staphylococcus species: The optimum nitrogen source for maximum dextransucrase production was estimated by incubating production medium M-3 containing 2%w/v Sucrose (pH 6.0) and different nitrogen sources in 5flasks (Yeast extract 0.5%, Beef extract 0.5%, Peptone 0.5%,

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Tryptone 0.5%, BHI 0.5%) with 4%v/v inoculum for 24 hours at 370C (150rpm). Dextransucrase activity for all different nitrogen sources was estimated by the DNSA method.

5.6 Optimization of the effect of different Carbon sources on dextransucrase production by Staphylococcus species: For optimizing the effect of different Carbon sources on dextransucrase activity, the production medium M-3 with different carbon sources (1%w/v) Glucose, Sucrose, Maltose, Lactose, and Control was incubated with 24 h old seed inoculum (4%, v/v) at 37^0 C (150rpm) for 24 h and then tested by DNSA method for maximum dextransucrase activity.

5.7 Optimization of Incubation Time for maximum dextransucrase production by Staphylococcus species: Optimization of the effect of different incubation time periods (after 24 hours, after 48 hours, after 72 hours) was estimated by incubation of selected bacterial strain into M-3 medium containing 2 % sucrose (pH 6.0). Then estimation of enzyme activity was done by DNSA method.

6. Production and purification of Dextran by crude enzyme dextransucrase:

6.1 Production and estimation of Dextran by Phenol-sulfuric acid method: For the production of Dextran, Firstly dextransucrase was produced by incubation of production medium M-3 under optimized conditions. Then crude enzyme product was obtained by centrifugation at 10,000 g for 10 minutes. The supernatant (0.25ml) was used as crude enzyme for inoculation of 50ml (2% Sucrose solution) prepared in 15mM Sodium acetate buffer (pH 6.0). This was incubated for 96 hours in an incubator shaker at 150rpm. The amount of dextran produced after 24 h, 48 h, 72 h, and 96 h was tested by the Phenol- sulfuric acid method.

6.2 Purification of Dextran: After completion of the production process of dextran, purification was done by using an equal amount of 75% ethanol for the precipitation of dextran from the sample. The sample precipitate was re-suspended in water and treated with 25% TCA to remove all protein content. Ethanol precipitation was done twice to remove all impurities. The purified dextran was then re-dissolved in Water and freeze-dried to get its powder form.

7. Characterization of Dextran by solubility and viscosity testing:

7.1 Solubility of Dextran: The powered dextran in small quantities was taken and poured into five different solvents, Distilled Water, Glycerol, Polyethylene glycol, Methanol, and Ethanol. Then powered dextran in each solvent was vortex for 30 sec. and the results were observed.

7.2 Viscosity of Dextran: The powdered dextran 0.1%, 0.2%, 0.3%, 0.4%, and 0.5% was dissolved in Distilled water, and then its flow time was determined with the help of a

Viscometer. After that, for each dextran concentration, the gravity was checked with the help of a specific gravity bottle. The values of Viscosity for all concentrations were calculated by the Viscosity formula with values of known (standard) density and Viscosity of Distilled water at Room temperature.

Results and Discussion:

1. Isolation of dextransucrase-producing bacteria from curd sample: White colonies were obtained from MRS agar medium which was further streaked on the same medium for obtaining pure culture.

2. Selection of isolate having higher dextransucrase activity: Isolate having higher dextransucrase enzyme activity was selected from a total of 4 pure culture isolates (Table 1.3 and Figure 2).

S.NO	ISOLATE NUMBER	ENZYME ACTIVITY(U/ml)
1	S1	0.103
2	S2	0.278
3	S3	0.626
4	S4	0.023

Table 1.3 Four dextransucrase-producing isolates isolated from different curd samples. (Source: Primary)

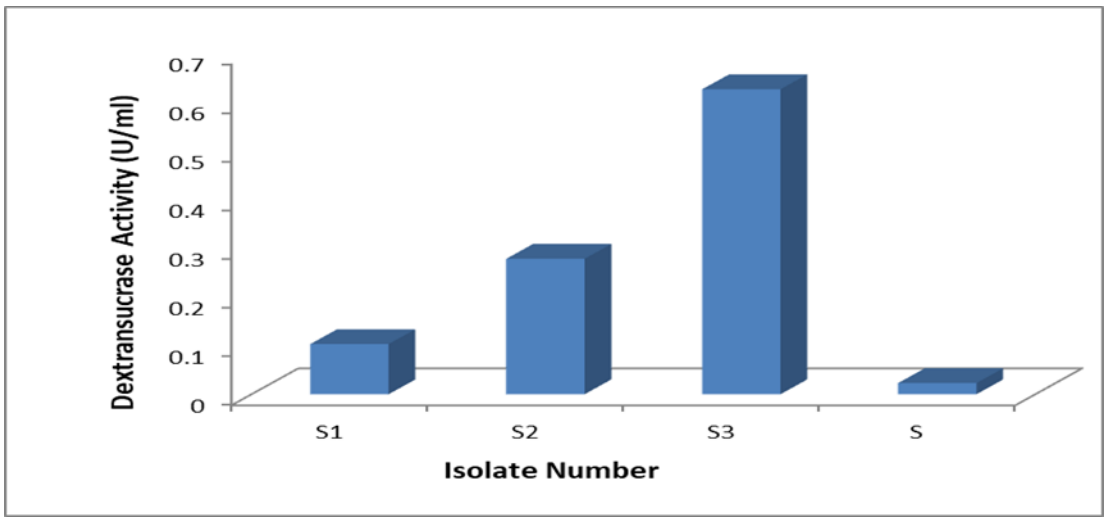

Figure 2.Selection of Isolate having maximum dextransucrase Activity (Source: Primary)

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3 Identification of isolate: Dextransucrase hyper producer isolate 3 showed maximum enzyme activity and was identified according to Bergey's manual of determinative Bacteriology. The isolate was identified up to the genus level using ABIS online laboratory tool by morphological and biochemical characterization viz., Grams staining, Indole test, Methyl red test, Voges-proskauer test, Citrate utilization test, Carbohydrate fermentation test, Catalase test, Oxidase test and Hydrolysis of casein, starch and urea.

Figure 3.Identification of isolate no. 3 as Staphylococcus sp. by ABIS online laboratory tool. (Source: Primary)

4. Selection of media for dextransucrase and Optimization of variour parameters for maximum dextransucrase production: The culture was grown on Five different media's which were reported for dextransucrase production (table 1.2).Out of these five mediums, M-3 medium containing Sucrose (5%), Tween 80 (0.1%), Yeast-extract (1.5%), Peptone (2.0%), K2HPO4 (1.5%) was suitable for dextransucrase production. The maximum dextransucrase activity was obtained by this medium (0.696 U/ml) as shown in Figure 4. Moreover, optimum parameters such as effect of different sucrose concentration in selected medium, effect of pH level in selected medium, inoculum size, effect of differet nitrogen sorces, effect of different carbon sources, incubation time etc. The parameters for maximum dextransucrase activity were selected as shown in different figures(5, 6, 7, 8, 9, 10).

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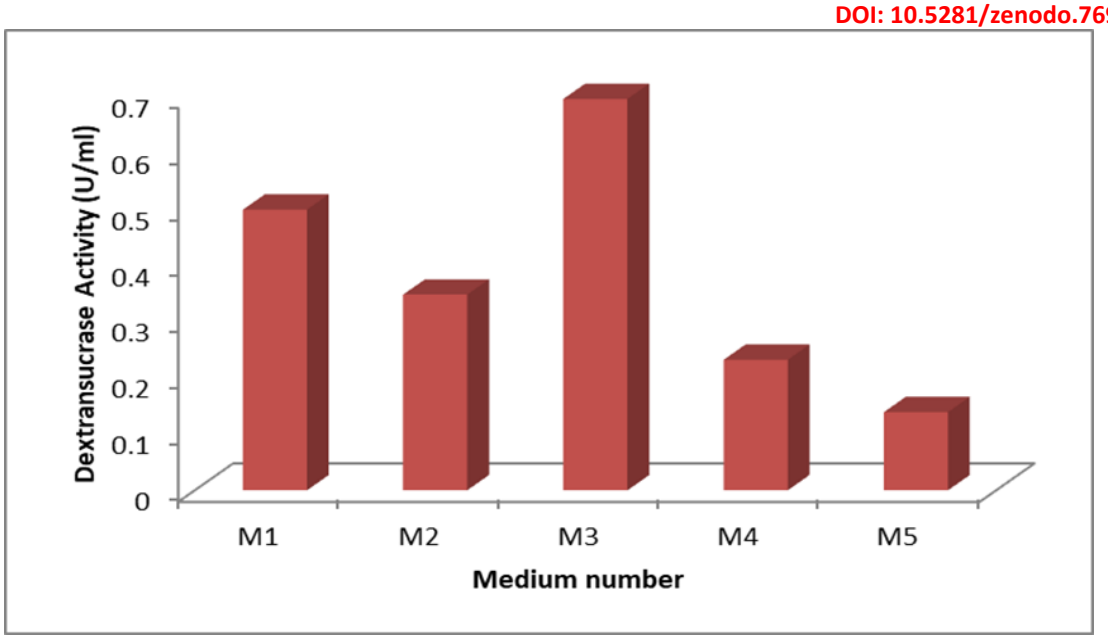

Figure 4. Selection of medium for maximum dextransucrase activity by Staphylococcus sp.(Source: Primary)

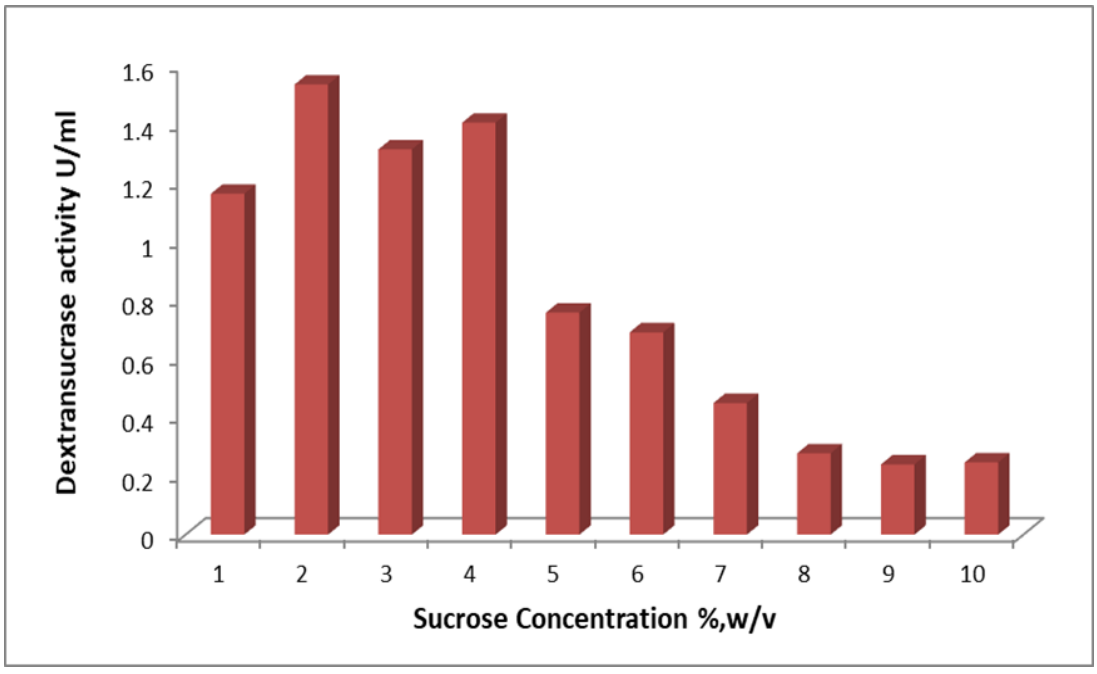

Figure 5.Optimization of sucrose concentration for maximum dextransucrase activity by Staphylococcus sp. (Source: Primary)

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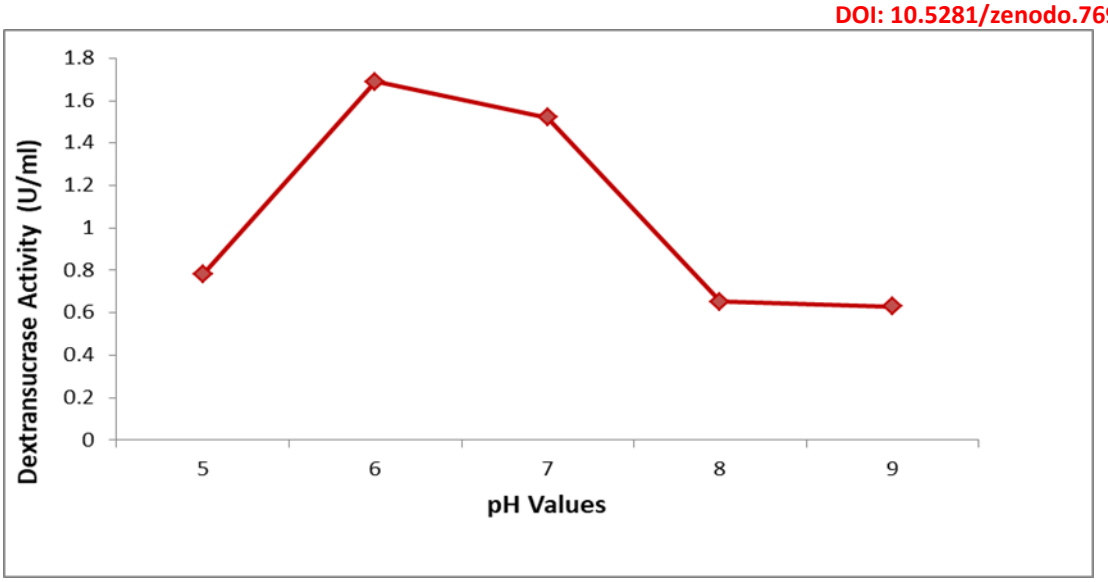

Figure 6.Optimization of pH for maximum dextransucrase activity by Staphylococcus sp. (Source: Primary)

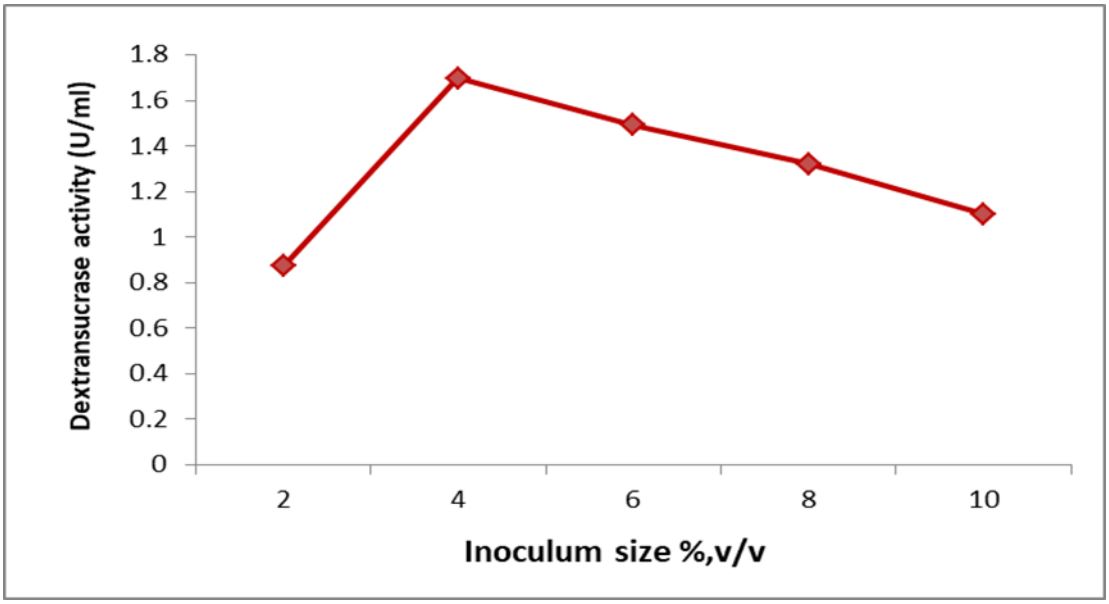

Figure 7.Optimization of Inoculum size for maximum dextransucrase activity by Staphylococcus sp. (Source: Primary)

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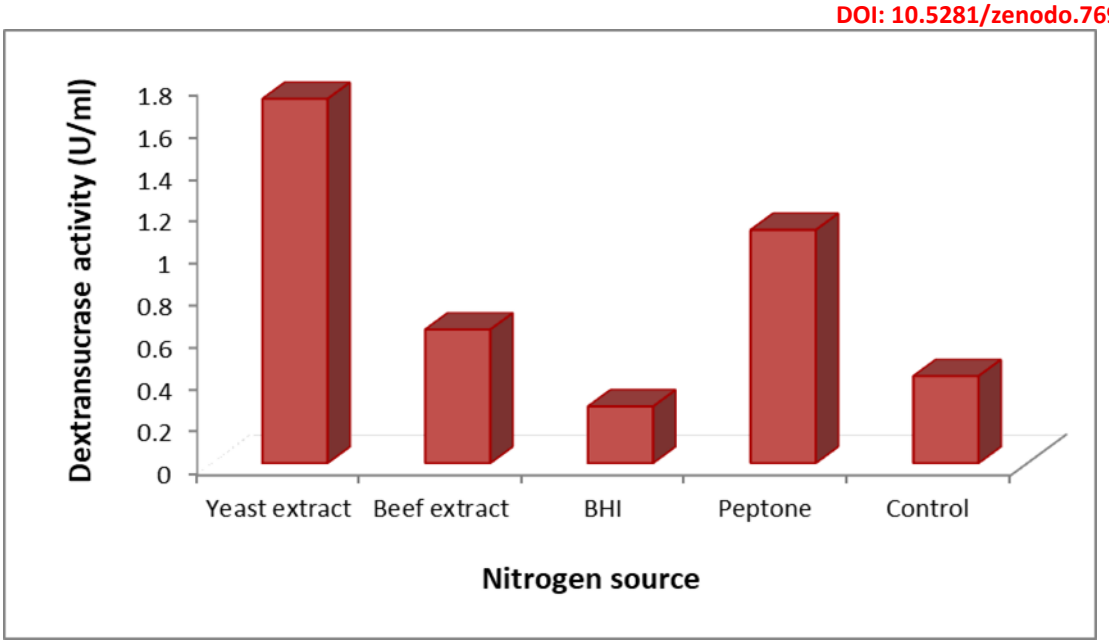

Figure 8.Optimization of nitrogen source for maximum dextransucrase activity by Staphylococcus sp. (Source: Primary)

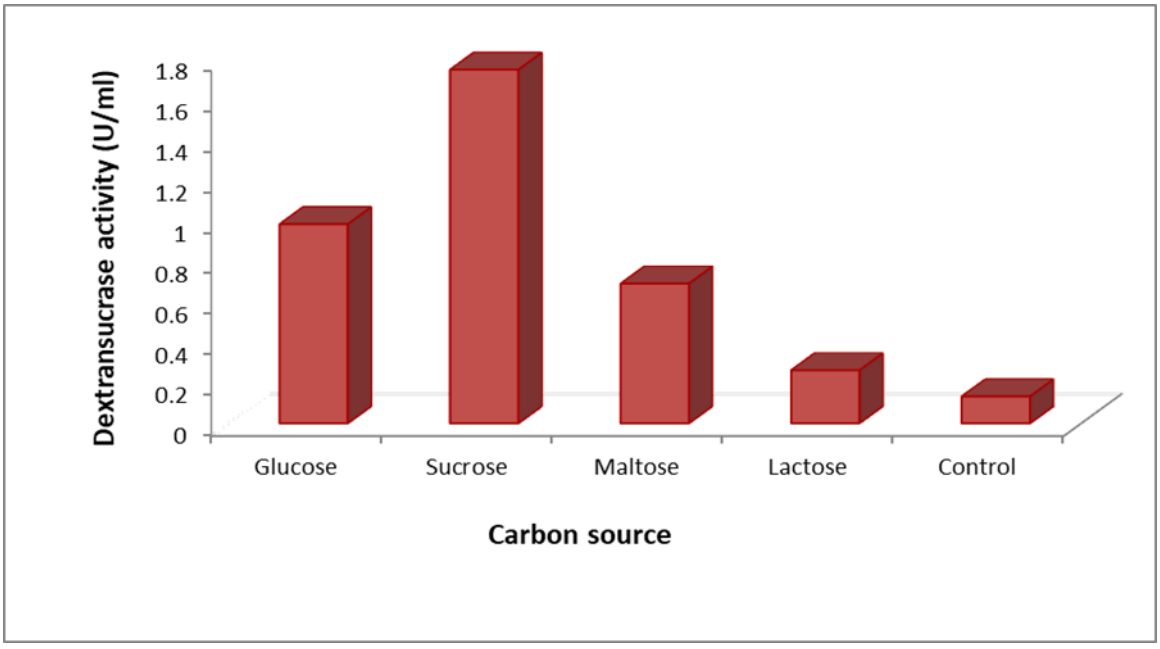

Figure 9.Optimization of Carbon source for maximum dextransucrase production by Staphylococcus sp. (Source: Primary)

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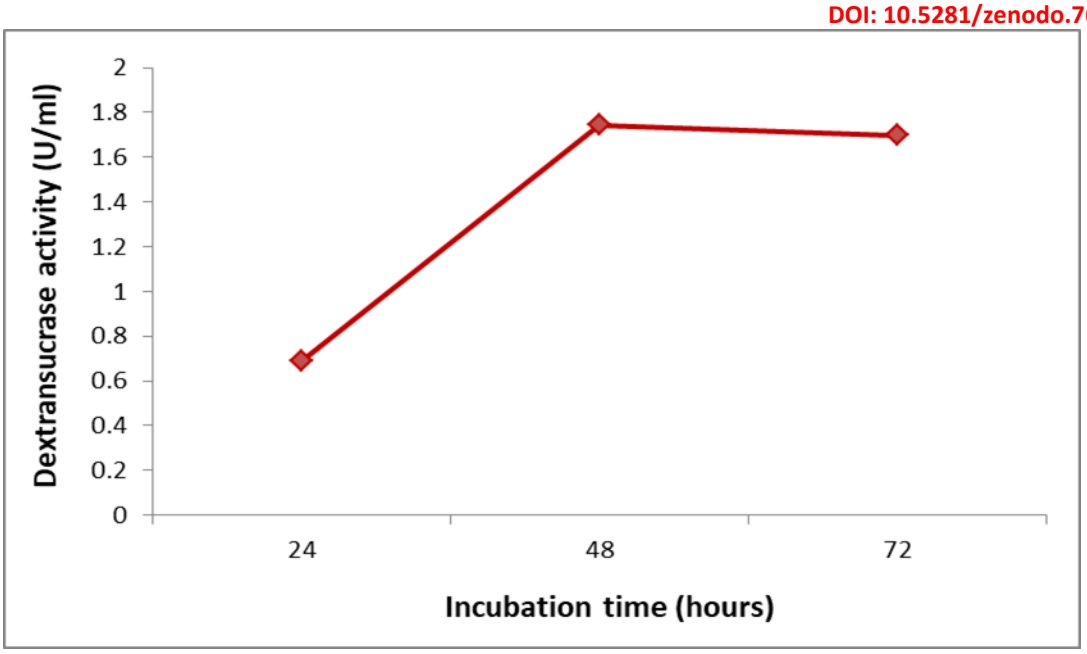

Figure 10.Optimization of Incubation time for maximum dextransucrase activity by Staphylococcus sp. (Source: Primary)

5. Production and purification of dextran from crude enzyme sample of dextransucrase of Staphylococcus sp.: For production of dextran, dextransucrase was produced by incubation of 24hours old seed medium into M-3 production medium at optimum culture conditions. The Supernatent obtained by centrifugation (10,000 g for 10 minutes) was used for further production of Dextran. Dextran was produced by incubation of 2ml dextransucrase in 400ml of 2% Sucrose Solution prepared in Sodium acetate buffer (25mM, pH 6.0). The incubation was carried out for 72 hours at 370C for maximum dextran production. After that dextran was precipitated with equal amount of 75% ethanol by centrifugation at 4,000 g for 30minutes.Supernatent was discarded and again ethanol precipitation was done. Dextran precipitate was then dissolved in distilled water and then treated with 5ml of 25% TCA for all protein removal. The purified Dextran precipitate obtained was again dissolved in distilled water and lyophilized to get its powder form (Figure 11).

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Figure 11. Purified dextran in powder form after lyophilization.(Source: Primary)

6. Estimation of dextran produced by dextransucrase of Staphylococcus sp.: The purified dextran sample after 24 h, 48 h, 72 h and 96 h of incubation was estimated. The maximum dextran production was estimated after 72 hours of incubation (0.659 mg/ml) as depicted in (Figure 12)

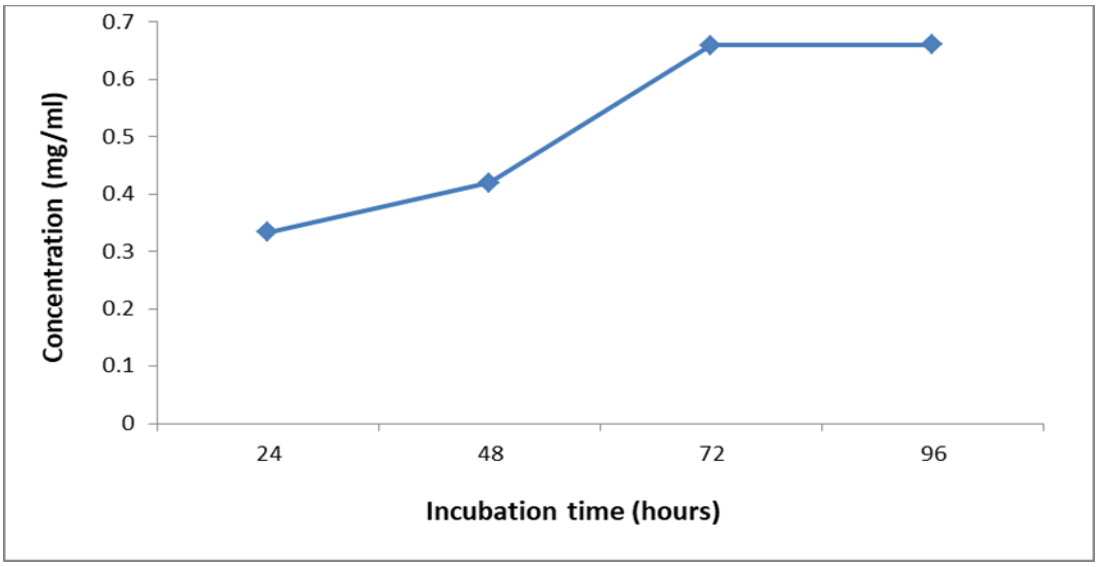

Figure 12.Estimation of Dextran after different intervals of time. (Source: Primary)

7. Characterization of Dextran produced: The dextran powder was then tested for its solubility in distilled water, glycerol, polyethylene glycol, methanol, and Ethanol. The dextran was completely dissolved in distilled water and glycerol but partially dissolved in polyethylene glycol, but insoluble in ethanol and methanol. Similar results were tested for dextran solubility

(Wamik, 2018). The viscosity for dextran was tested in concentrations 0.1%, 0.2%, 0.3%, 0.4%, and 0.5% (w/v) with the help of a viscometer. The viscosity of dextran increases, as the concentration of dextran increases. The calculated viscosity was shown in (table 4.3 and Figure 4.13).

S.NO.	Concentration % (w/v)	Viscosity (η), cP
1	0.1	0.896
2	0.2	0.909
3	0.3	0.931
4	0.4	0.981
5	0.5	1.03

Table 1.4.Viscosity of Dextran in different concentrations (%,w/v) (Source: Primary)

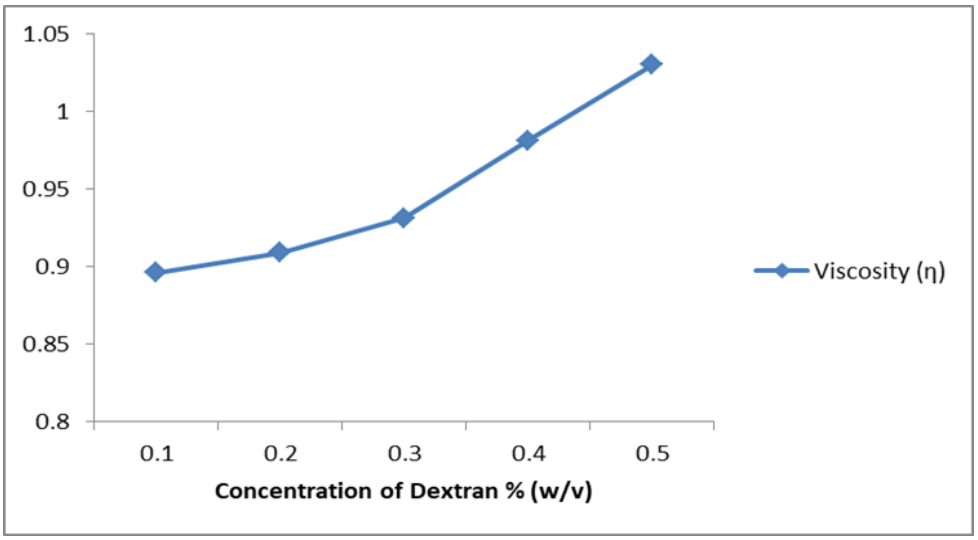

Figure13.Optimization of viscosity (η) of Dextran. (Source: Primary)

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