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A STUDY ON SCREENING ,OPTIMIZATION, PRODUCTION AND APPLICATION OF DEXTRAN FROM WEISSELLA CIBARIA

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Abstract: Dextran a high molecular weight exopolysaccharide with significant industrial and biomedical applications is predominantly produced by latic acid bacteria. This study focuses on the screening, optimization, production, and application of dextran from *Weissella cibaria* a promising dextran production microorganism. Intially various *Weissella cibaria* were screened for dextran production using sucrose based media with W. cibaria demonstrating superior yield and polymer quality. Optimization of culture condition such as pH, temperature, incubation, time and sucrose concentration was carried out using Response Surface Methodology (RSM) to maximize dextran yield. The optimized condition resulted in a significant enhancement of dextran production, confirmed through gravimetric and spectrophotometric analyses. Structural characterization via FTIR and NMR confirmed the polymeric nature and functional groups of the dextran produced. Finally the application potential of the produced dextran was evaluated in food and pharamaceutical contexts, particularly as a prebiotic and a stabilizing/thickening agent. The study demonstrates the feasibility of using W. cibaria as a sustainable microbial source for high quality dextran with potential for scalable industrial production.

I.INTRODUCTION:

Dextran is a complex, high-molecular-weight exopolysaccharide (EPS) composed of glucose units.It is produced by various lactic acid bacteria (LAB), including members of the genus *Weissella*, which are commonly found in fermented foods. Dextran is particularly interesting due to its unique physical and chemical properties, such as high water solubility, biocompatibility, and biodegradability. These characteristics make it a valuable compound with a wide range of applications in various industries. *Weissella cibaria*, a Gram-positive bacterium, has been identified as a highly efficient producer of dextran. The production of dextran by this bacterium is a metabolic feature that is influenced by several factors, including the composition of the culture medium and the environmental conditions of fermentation.

EXOPOLYSACCHARIDES

Polysaccharides are polymeric carbohydrate molecules composed of long chains of monosaccharide units bound together by glycosidic linkages.Some of the important storage polysaccharides are starch,glygogen.Structural polysaccharides are cellulose and chitin, they are naturally present in plants and animals. Exopolysaccharides are natural polymers of high molecular weight compounds secreted by microorganisms into their environment.It is abbreviated as extracellular polymeric substances(EPSs) and mostly composed of proteins and macromolecules such as DNA,lipids and humic substances. Microbial exopolysaccharides are class of Biothickners that are found in two forms of capsules and slime.Dextran,xanthan,gellan are the examples of industrially important microbial exopolysaccharides (Moosavi-Nasab *et al.,*2009).

SOURCES OF EXOPOLYSACCHARIDES

- **Plants**: Cellulose,Pectin
- **Algae**: Agar,Alginate,Carrageenan
- **Bacteria**: Alginate,Dextran,Pullulan,Xanthan gum(Tapan, 2012)

Dextran is a bacterial polysaccharide which is biochemically a branched glucan made up of glucose molecules .It is produced by as low and high molecular weight dextrans(from 10 to 150 kilo Daltons). It is an extracellular bacterial polymer of D-glucopyranose with predominantlyα-(1→6) linkage in the main chain(Monsan *et al.,* 2001).

PROPERTIES OF DEXTRAN

The molecular weight of dextran is 1,000 Daltons from 2,000,000 Daltons.It is readily soluble in water and electrolyte solutions.It is also soluble in solvents such as methyl sulfide,formamide, ethylene glycol, glycerol and insolublein alcohol. Different carbon sources like sucrose, glucose, maltose and lactose are used for the dextran and dextransucrase. Dextransucrase is an extracellular enzyme produced by dextran producers.

APPLICATION OF DEXTRAN

Food industry

Dextran is the first industrial polysaccharide which was produced by lactic acid bacteria.It improves moisture retention,viscosity and inhibits sugar crystallization.It acts as jelling agents in gum and candies. It also acts as a food preservatives which inhibit the growth of spoilage micro organisms(Whistler *et al.,*1990).

Photographic industry

- It improves the quality of silver emulsion of photographs.

Waste water management

- Dextran can be stable in alkali and acidic conditions.It binds to metal ions at alkaline pH.So it is used in waste water treatmentduring the flocculation process.

Cosmetics application

- Cationic Dextran is used in hair care and skin care products. Dextran sulfate has different properties such as anti-ageing, anti-wrinkle effects, good moisture retention, anti-inflammatory, anti-allergic.

Biomedical application

Dextran used as a drug especially blood plasma volume

expander,iron carrier or anticoagulant in pharmacy. Cross linked dextran sephadex is used in research and industry for separation and purification of Protein (Phimchanok*et al*.,2016).

Anti-thrombotic effect

It is used to decrease the vascular thrombosis.The antithrombotic effect of dextran is synthesized by its binding of erythrocytes, platelets and vascular endothelium, increasing their electro-negativity reducing erythrocytes aggregation and platelet adhesiveness.

Used in intravenous fluids

Dextran in intravenous solution provides a neutral fluid once in the body is digested by cells into glucose and free water.In emergency situations it is used to replace the blood loss.

Anti-coagulant activity

Clinically prepared dextran has the anti-coagulant activity;it is the safest plasma substitute.It also used in cryopreservation, solutions for storing organs for transplantation and act as carrier in vaccines.

II.AIM AND OBJECTIVE

The present study is taken-up with the following objectives

- To isolate Dextran producing bacteria from a natural source.
- To identify the isolate by biochemical and molecular characterization (16S rRNA sequencing).
- To optimize the various parameters for the production of dextran.
- To apply the partially purified dextran for thrombolytic activity.

III.MATERIALS AND METHODS

SAMPLE COLLECTION

Sugarcane juice in a sterile container wascollected from juice shop in Karur District, Tamil Nadu, India for the isolation of dextran producer.

ISOLATION OF ORGANISM FROM SUGARCANE JUICE

- Collected sample was inoculated in broth containing sucrose - 5g, Tryptone -1g, Yeast extract- 0.5g, K_2HPO_4 - 1.25g, pH- 7.0 for the enrichment.
- Prepared medium was autoclaved it for 121ºC for 15 mins and then added 0.005% sodium azide. About 1ml of sample was inoculated and incubated at 25ºC for 24 hours with vigorous shaking (150 rpm)
- After incubation, uniform turbidity was seen and one loop full of inoculums was taken and streaked on sucrose agar plat. Incubated the plates at 25ºC for 24 hours. After incubation colonies with mucoid, shiny slime layer and round in shape was selected and sub-cultured on MRS agar for every 4 days.

IDENTIFICATION BY BIOCHEMICAL CHARACTERIZATION

Gram's Staining

The smear was prepared in the clean glass slide. It was dried and heat fixed. It was stained with basic dye crystal violet for 45 seconds(Solution A crystal violet (90%dye content)- 2.0g dissolved in Ethyl alcohol (95%)- 20.0ml and solutions are mixed).The stain was washed off with distilled water.The smear was followed by treatment with an iodine solution. Iodine solution was removed by gently added with distilled water. The smear was decolorized by 95% ethanol. Then it was counterstained with saffranin for 30 seconds.The slide was dried and observed smear under the microscope.

Indole Test

Peptone water tubes were prepared. The isolates were inoculated into the peptone water tubes.The tubes were kept for incubation at 37ºC for 24 hours.After the incubation period 2-3 drops of KOVAC' S reagent was added and observe the results.

Methyl Red (MR) Test

Acid production of isolates was detected by using MR-VP broth (peptone -7.0g, potassium phosphate -5.0g, dextrose - 5.0g, distilled water – 1000ml and pH 6.9) was prepared and sterilized at 121ºC for 15 minutes.The isolates were inoculated into sterilized MR-VP broth and incubated at 37ºC for 24-48 hours. After the incubation period 2-3 drops of methyl red reagent was added and observe the results.

Voges- Proskauer (VP) Test

Non acidic end product was detected to the isolates by using MR-VP broth. MR- VP broth was prepared and sterilized of 121ºC for 15 minutes.The isolates were inoculated and incubated at 37ºC for 48 hours. After the incubation period 2-3 drops of Barrit's reagent A and B were added into the culture tubes and mixed thoroughly before recording result.

Citrate Utilization Test

Simmons citrate agar was used to detect the ability of citrate utilization by the isolates. Simmons citrate Agar(SCA) medium(Ammonium dihydrogen phosphate – 1.0g, dipotassium hydrogen phosphate -1.0g, sodium chloride – 5.0g, sodium citrate- 2.0g, magnesium sulphate- 0.2g, bromothymol blue- 0.08g, agar- 18.0g, distilled water- 1000ml and ph- 6.9) was prepared and sterilized at 121ºC for 15 minutes.The isolates were streaked on the agar slants and incubated for 18- 24 hours at 37ºC. After the incubation period the results were observed.

Urease Test

Christenson Urea agar was used to detect the urease enzyme production by the isolates. Christenson Urea agar medium (peptone from meat- 1.0g, D(+) glucose- 1.0g, sodium chloride- 5.0g, potassium dihydrogen phosphate- 2.0g, phenol red -0.0012g, urea- 20.0g, agar- 12g, distilled water- 1000ml and ph- 6.8) was prepared and sterilized at 121ºC for 15 minutes. The isolates were streaked and incubated for 13-24 hours at 37ºC. After the incubation period the results were observed.

Triple Sugar Iron Test(TSI)

TSI medium was used to detect the ability of the test organisms to ferment Glucose, Lactose, Sucrose and H_2S production. TSI medium (beef extract-3g, Yeast extract- 3g, peptone- 15g, sodium chiorite-5g, glucose- 1g, sucrose- 2g, protease peptone- 5g, distilled water- 1000ml and ph- 6.8) was prepared and sterilized at 121ºC for 15 minutes. The isolates were

streaked on the TSI agar slants and incubated for 13- 24 hours at 37ºC. After the incubation period the results were observed.

Carbohydrate Fermentation Test

It is used to detect the ability of the test organisms to ferment Glucose, Sucrose, Lactose and Maltose.The medium contains (peptone- 1g, NaCl – 0.5g, sugars – 1g, phenol red indicator- 0.720µl, distilled water- 100ml)was prepare and sterilized at 121ºC for 15 minutes.The isolates were inoculated and incubated for 24 hours at 37ºC.After the incubation period the results were observed.

SPECIES IDENTIFICATION BY 16Sr RNA SEQUENCING

DNAExtraction

- Cells grown in agar plater was lysed by suspending 1-3 coloniesaseptic allyandmixedwith450µlof"BCube"lysisbufferina2mlmicro centrifuge tube and lyse the cells by repeated pipetting. Added 4 µl of RNAse A and 250 µl of "B Cube" neutralization buffer.
- Added 4 µl of RNAse A and 250 µl of "B Cube" neutralization buffer. Vortexed the content and incubated for 30 minutes at 65˚C in water bath. To minimize shearing the DNA molecules mixed DNA solutions byinversion.
- The tubes were centrifuged for 15 minutes at 14,000 rpm at 10˚C. Following centrifugation, transferred the resulting viscous supernatant into a fresh 2ml micro centrifuge tube without disturbing the pellet.
- Tothe above content added600µlof"BCube"binding buffer and mixed thoroughly by pipetting and incubated at room temperature for 5minutes.
- About 600 µl of the content were transferred to a spin column placed in 2 ml collectiontube. Centrifuged for 2 minutes at 14,000 rpm and discarded flow-through
- Reassembled the spin column and the collection tube then transferred the remaining 600 µlof thelysate. Centrifuged for 2 minutes at 14,000 rpm and discarded flow-through.
- About 500 µl "B Cube" washing buffer I was added to the spin column, centrifuged at 14,000 rpmfor 2 mins and discardedflow-through
- Reassembled the spin column and added 500 µl "B Cube" washing buffer II centrifuged at 14,000 rpm for 2 mins and discarded flow-through
- DNA concentrations were measured by running aliquots on 1% agarose gel.
- The DNA samples were stored at -20°C until further use.

PCR Protocol

Polymerase Chain Reaction (PCR) is a process that uses primers to amplify specific cloned or genomic DNA sequences with the help of a very unique enzyme. PCR uses the enzyme DNA polymerase that directs the synthesis of DNA from deoxynucleotide substrates on a single-stranded DNA template. DNA polymerase adds nucleotides to the 3` end of a custom-designed oligonucleotide when it is annealed to a longer template DNA.

Composition of the Taq Master Mix

- Taq DNA polymerase is supplied in 2X Taq buffer, 0.4mM dNTPs, 3.2mM $MgCl_2$ and 0.02% bromophenol blue

Primer details

Forward - AGAGTTTGATCMTGGCTCAG
Reverse – TACGGYTACCTTGTTACGACTT

About 5 µl of isolated DNA was added in 20 µl of PCR reaction solution (1.5 µl of Forward Primer and Riverse Primer, 5 µl of deionized water, and 12 µl of Taq Master Mix). Performed PCR using the following thermal cycling conditions.

- Denaturation for 94°C for 30 sec,
- Annealing for 50°C for 60 sec,
- Renaturation for and 72°C for 60 sec.

Purification of PCR Production

Removed unincorporated PCR primers and dNTPs from PCR products by using Montage PCR Clean up kit (Millipore).The PCR product was sequenced using the 27F/1492R primers. Sequencing reactions were performed using a ABI PRISM® BigDyeTM Terminator Cycle Sequencing Kits with AmpliTaq® DNA polymerase (FS enzyme) (Applied Biosystems).

PRODUCTION OF DEXTRAN

- Culture medium containing Sucrose -15g,Peptone – 0.5g, Yeast extract – 0.5g,K_2HPO_4 – 1.5g, $MnCl_2.H_2O$ – 0.001g, NaCl – 0.001g, $CaCl_2$ – 0.025g. pH- 7.0 was prepared and sterilized at 121ºC for 15 mins. 10ml of overnight mother inoculum was inoculated in 90ml of freshly prepared production medium. The flask were incubated at 30ºC for 24 hours in a shaker (150 rpm)

PRECIPITATION OF DEXTRAN

- After 24 hours equal amount of chilled ethanol was added into the culture broth and shaken vigorously. Then centrifuged at 10,000 rpm for 15 mins and supernatant was decanted. Repeated this step for twice a time. Precipitated dextran was dried at 30ºC and the percentage of crude dextran was calculated.

PURIFICATION OF DEXTRAN

- To remove the impurities the dextran was dissolved in distilled water and then dextran slurry was again precipitated with chilled ethanol. Dissolving, precipitation and washing was repeated for thrice to remove the cell debris. Then the purified dextran was dried at 30ºC and the percentage of yield was calculated

THROMBOTIC ACTIVITY OF PURIFIED DEXTRAN

- About 2.5ml of venous blood was collected and distributed

in 5 different pre weighed sterile microcentrifuge tube (0.5ml/ tube).

- The tubes were incubated at 37ºC for 45mins.
- After clot formation, each tube having clot was weighed to determine the clot weight (clot weight of clot containing tube – weight of the tube alone).
- One percentage dextran solution was preparedby dissolving 1g of purified dextran in 100ml PBS.
- About 50 – 200µl of 1% dextran solution was added to the tube containing clot. Commercially available lyophilized Streptokinase and 2.5ml of phosphate buffer solution was added and mixed well, which was kept as positive control.
- For negative control 100µl of distilled water was added.
- Then the tube was incubated at 37ºC for 90mins and observes the clot lysis.
- After incubation, the fluid was removed and the tube was weighed again.
- Difference obtained in weight taken before and after clot lysis was expressed as percentage of clot lysis.
- Percentage of clot lysis = weight of the clot after lysis by sample × weight of the clot before lysis.

IV.RESULT& DISCUSSION

Organism was isolated from sugarcane juice and further subjected for identification by using various test, such as microscopy and biochemical tests.The results of biochemical tests of the isolate indicated that the isolate was Gram-positive, non-motile, rod shape bacteria. It was positive for oxidase, negative for catalase, citrate utilization, whereas negative for indole, MR and VP. It produced acid end product by fermenting glucose, sucrose and negative for all other sugars. Based on biochemical properties the isolates remained close resemblance to *Weissella* sp. even if, they illustrate variations in few tests.

In Gram staining, the slide was observed under the oil immersion (100 X) as purple color short rod appeared, so it identified as Gram positive bacteria (Plate 2). Bacterial colonies having cytochrome activity develop a deep blue colour at the inoculation site within 10 seconds. So it was indicated as Positive. No bubbles were formed so it was recorded as Negative. All other biochemical test results are shown in the table 1.

MOLECULAR IDENTIFICATION

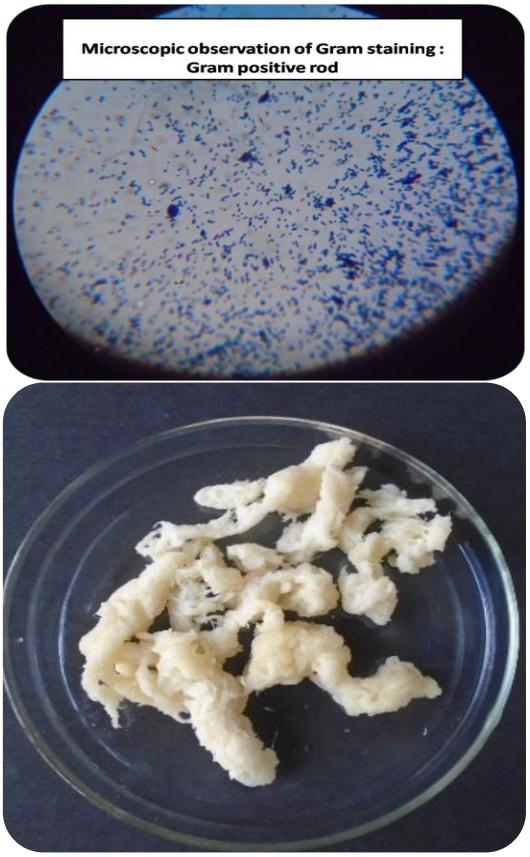
The genomic DNA of the isolate was isolated and checked on agarose gel and was found to be of high molecular weight and intact. The DNA was subjected to 16S rRNA gene amplification for identification. PCR product was purified and commercially sequenced. The 16S rRNA sequence of the pathogen was subjected to BLAST using mega blast tool of GeneBank (www.ncbi.nlm.nih.gov) and confirmed the identity of the bacterial isolates as *Weissella cibaria*. It exhibited maximum 100% homology with other pseudomonas species. The 16S rRNA gene sequences of these strains have been deposited in the GeneBank and awaiting for accession number.

AMPLIFIED 16S rRNA SEQUENCES OF THE TARGET ISOLATE

TCATCTGTCCCACCTTGGACGCCTGCCTCCTGAAGGTTA
CCCCACCGACTTGTGACACACTCTCATGGTGTGACGGG
CGGGGTGTACCAGACCGGGGAACGTATTGGCGTTCTGA
TCCACGATTACCAACGATTCCGACTTCAGGTAGCCGAG
TTGCAGCCTCCGGCTACGACGAACTTTATGAGATTAGCT
CCACCTCGCGGCTGGCCCACTTGTGCCATTGTAGCGCGT
GTGTCGCCCATGTCATAAGGGGCATGATGATTTGACGT
CATCCTTCC
TCCGGTTTGTCCCCGGCAGTCTCACTAGAGTGCCGAACT
ATATGATGGCGAAATAAGGGTTGCGCTCGGGGCGCTTC
TTAACCCAACATCTCACAACGGGGGCTGACCATGCACC
ACCTGTCACCTTGGCCCAGGAGGGAACGCCCCGTCTCA
GGAGTTGCAGGTCAAGAACTGGTAAGGTTTTTCGCGTT
GGAGTCGAATTAAACCATATGGCCGGTGTGCGGATGCA
GCAACATTTTTATTGAGGTTCAACCCTTGCGAGCGAAA
CCCCCAAATGAAGTAC TTAATGCGTAACTGCGGC

Result:*Weissella cibaria*

Precipitated Dextran



V.SUMMARY AND CONCLUSION

The present study was done to investigate the dextran producing *Weissellacibaria* from sugarcane juice. The samples were collected from juice shop in Karurdistrict Tamil Nadu, India.Then the sample was processed for the isolation and identification of the organisms. The species identification was confirmed by 16Sr RNA Sequencing as *Weissella cibaria*. Then the isolates were screened and optimization fordextran

production.In the present study, the dextran production was done in various parameters such as sucrose, yeast extract, pH, temperature, incubation period and NaCl with various concentration and time. Here sucrose(20g) showed the highest yield as 8.04g dextran. Yeast extract (0.3g) showed the highest dextran yield as 8.79g. At pH 6.5, it showed the highest dextran yield is 8.02g. Temperature 27ºC showed the highest dextran yield as 8.51g. Incubation period 17hrs showed the highest dextranyield as 8.42g. Then the NaCl (0.01g) showed the highest dextran yield as 7.25g.

VI.REFERENCE

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