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Research Paper

Comparison of Levansucrase production by *Serratia marcescens* BT47under SmF and SSF.

MINI K. PAUL, RASIJA RAJAN
Department Of Biosciences, Mes College, Marampally, Aluva-7, Ernakulam, Kerala, India.

ABSTRACT
Bacteria which synthesize levansucrase was isolated from decaying watermelon in this study. Only 32 of the 126 isolateshad a clearance zone of more than 0.2 cm, with 10 getting a defined zone of more than 0.5 cm. Secondary screening was performed on these ten isolates, and production was assessed using the dinitro salicylic acid method. According to Bergey's Manual of Systematic Bacteriology and 16 S rRNA sequencing, the highest levansucrase-producing bacteria was identified as S. marcescens BT47.Serratia marcescens BT47levansucrase production was compared under submerged fermentation (SMF) and solid state fermentation (SSF). According to our findingsS. marcescens BT47levansucrase production was highest under SSF utilizing tamarind shell powder as a substrate. There are no other reports of S.marscescens which produces levansucrase.

KEYWORDS: *Levansucrase, levan, Serratia marcescens BT47.Tamarind Shell,SSF,SMF*

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I. INTRODUCTION

Levansucrases (β-2, 6-fructan: D-glucose-1-fructosyltransferase, E.C.2.4.1.10) belong to the glycoside hydrolase family 68, and catalyzes the synthesis of levan molecules. Levan is a fructose polymer with β2–6 bond in main chain and branching in β2–1 links [1]. Levansucrase hydrolyzes sucrose to release glucose and transfer fructose molecules to a growing levan fructooligosaccharide (FOS) chain [2]. Levan is found in a small number of plant species and a wide spectrum of microbial products in nature. Because of their high output and better water solubility, microbial levans are chosen over plant levans for commercial usage in the fields of food, medicines, and cosmetics. *Bacillus subtilis* [3], *Bacillus megaterium* [4], and *Bacillus licheniformis* [5] were among the microorganisms that peroduced levansucrase.

FOS is used in a variety of applications, including medicine, cosmetics, pharmaceuticals, the food sector, and agriculture [6-9]. Levans are employed as a viscosifier, stabiliser, emulsifier, thickening, encapsulating agent, surface finishing agent, carrier for taste and fragrances, and prebiotic agent in the food industry. Levan has been utilised as an anti-obesity agent, hypocholesterolemic agent, anticancer agent, hypolipidimic agent, antidiabetic agent, antiviral agent, and antipathogenic agent in pharmaceutical applications. Levan can be utilised as a bio-sorbent and bioflocculant in the environmental field.

Levansucrase has been studied extensively as a key biocatalyst in the production of leven and levan-type fructoolygosaccharides [10]. Despite the fact that various research on levan production have been published, all of them have low yields and impure product contamination.The goal of this study was to isolate and identify levansucrase-producing novel microorganisms in light of the application of levan by levansucrase and the prospect of isolating potential levansucrase-producing bacteria. The next goal was to compare levansucrase production by *S. marcescens* BT47under SMF and SSF conditions.

II. MATERIALS AND METHODS

2.1 Isolationof levansucrase producingbacteria

2.1.1 Samplecollection

Rotten watermelon was collected from different shopes located at Thattanpady and Marampally,Ernakulam,Kerala. Aseptically, the samples were carried in polythene bags to our microbiology lab.

2.1.2 Primary screening of levansucrase producingbacteria

Organisms isolated from watermelon were screened for levansucrase activity on media containing 10 % sucrose in agar plate. The fruits were squeezed and the juice is spread on the agar plate. The plates were incubated at 37^0 C for 2-3 days. Levansucrase producing bacteria were selected for further studies based on the zone of clearance around the colonies. The bacteria which showed zone of clearance of 0.5 cm or above around the colonies selected for testing the yield of levansucrase. The bacteria were sub cultured and maintained in agar slant containing 10% sucrose.

2.1.3 Secondary screening of levansucrase producingbacteria

For the preparation of inoculum, the bacterium was first grown on the sucrose agar slants for 48 hours. A loopful of growth was then transferred to sucrose broth and incubated at 37^0 C for 48 hours in an incubator shaker agitated at 100 rpm. After attaining the turbidity the culture was centrifuged at 5000 rpm for 10 min. at 4^0 C the supernatant was collected to estimate the levansucrase activity. Levansucrase activity was assayed by dinitrosalicylic acid (DNSA) method [11]. The activity was assayed in a reaction mixture containing 1 ml of suitably diluted enzyme extract and 10% of sucrose dissolved in 0.05 M phosphate buffer (pH -7) and incubated at 30^0 C for 30 minute. Maltose was released when it is heated. From this reaction mixture 1 ml was transferred to a clean test tube to which 2 ml of distilled water, 1 ml of DNS reagent and 2 ml of NaOH were added and keep in water bath for 10 minute. Absorbance was read at 540 nm. Concentration of maltose was quantified using a standard curve already prepared. One unit of levansucrase was defined as the amount of enzyme that liberated one micromole of maltose per ml per minute under the assay condition.

2.2 Identification of bacterium

2.2.1 Biochemical Identification

The cultural**,** morphological, physiological and biochemical properties of highest levansucrase producing bacteria were studied as part of identification. Identification was done according to the guidelines in *Bergey's Manual ofSystematic Bacteriology*[12]. The result was also confirmed by 16s r RNA gene sequence based molecular identification. For this isolation of genomic DNA and PCR were conducted as per methods described by Sambrook *et al* [13].9. A similarity search for the nucleotide sequence of 16s rRNA gene of the test isolate was carried out using a BLAST search at NCBI20. The sequence of forward primer (16 SF) and reverse primer (16 SR) used in the PCR amplification was and 5'-ACGGCTACCTTGTTACGACTT-3' respectively. The DNA sequencing was performed at the DNA sequencing facility of Sci Genome, Cochin. Forward primers were used to obtain the sequence of PCR product. A similarity search for the nucleotide sequence of 16s rRNA gene isolate was carried out using a BLAST search at NCBI. Stability of selected isolates is the ability to maintain high yielding nature was studied by sub culturing and testing the levansucrase yield at monthly intervals till the end of this work.

The same levansucrase producing bacterial strains isolated from rotten water melon mentioned under the section 2.1.3 were subjected to SSFand SmF studies.

2.3 Levansucrase production through SMF

The bacterial suspension was inoculated into 100 ml of the 1% sucrose broth (pH 6.5) and incubated at 37° C for 48 hours in an incubator shaker agitated at 100 rpm.The culture was centrifuged at 8000 rpm for 20 minutes and the supernatant was collected to estimate the chitinase activity

2.4 Levansucrase production through SSF

2.4.1 Preparation of substrate

Tamarind peels was collected from nearby areas of Marampally. The peel was then sun dried, milled and separated through different sized mesh.

2.4.2 Preliminary studies

The SSF experiment were carried out in 250 ml Erlenmeyer's flask using 5g of tamarind powder moistened with 10 ml distilled water. Cotton plugged flask were autoclaved at 121^0C under 15 lbs pressure for 15 minutes, cooled to room temperature and uniformly inoculated with 5ml inoculum of 72hour culture and incubated at 37^0 C for 4 days. After incubation the fermented substrate was mixed with 90 ml of distilled water on a rotary shaker for 30 minutes. The suspension was then centrifuged at 5000 rpm at 4^0 C for 10 minutes and the supernatant was assayed for enzyme activity. Levansucrase activity was assayed as mentioned under the section 2.1.3.

III. RESULTS

3.1 Isolation of levansucrase producing bacteria

A total of 126 levansucrase producing bacteria were isolated from different sample collected from different shops of Marampally. Among the 126 isolate only 32 produces zone of clearance over 0.2cm, in them 10 produces clear zone above 0.5cm. The isolate designated as BT47 produce highest zone of clearance. Those

bacteria which produce clear zone above 0.5 cm were subjected secondary screening. Levansucrase production (U/mL) was shown in table 1.

Table 1.Levansucrase production by top, high yielding strains

Strain	Levansucrase activity in the culture supernatant (U/mL)
BT6	19±1.12
BT8	26±1.82
BT13	27± 1.76
BT18	17±1.32
BT25	25± 0.65
BT36	30± 1.42
BT47	40± 0.32
BT68	30± 3.42
BT108	34± 1.54
BT112	27± 1.08

3.2. Identification of organism

The results of morphological and biochemical test of selected BT47 strain shown in the Table 2andFig.1-6 shows the figures of biochemical test results the figures of biochemical test results. As per the Bergey's Manual of systematic Bacteriologythe bacterium was identified as *Serratia.*

Table 2. Biochemical test

PRELIMINARY AND BIOCHEMICAL TESTS	
Tests	**Selected strain (T_7)**
Staining	Gram negative
Motility	Motile
Indole	Negative
MR	Negative
VP	Negative
Citrate	Positive
TSI	No H_2S
Urease	Positive

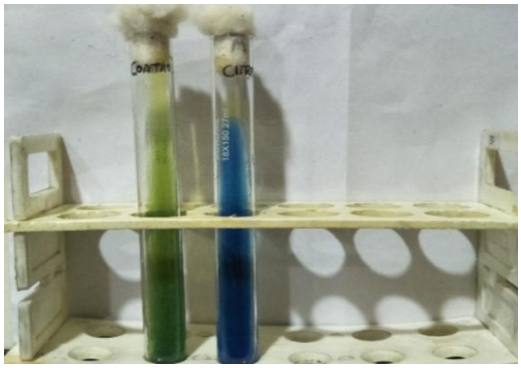

Fig. 1 Citrate test

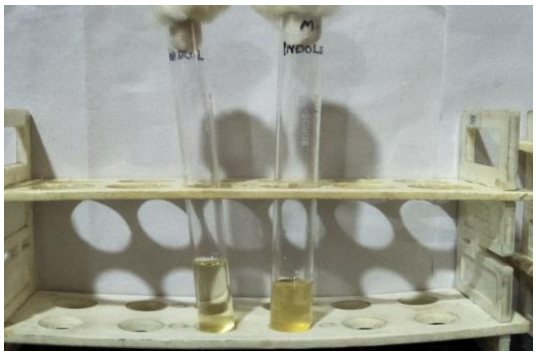

Fig.2 Indole Test

Fig. 3MR test

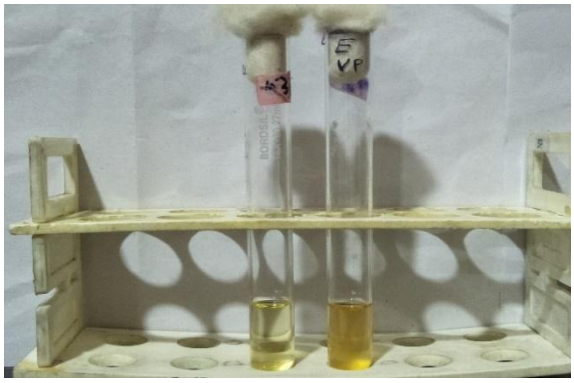

Fig. 4 VP

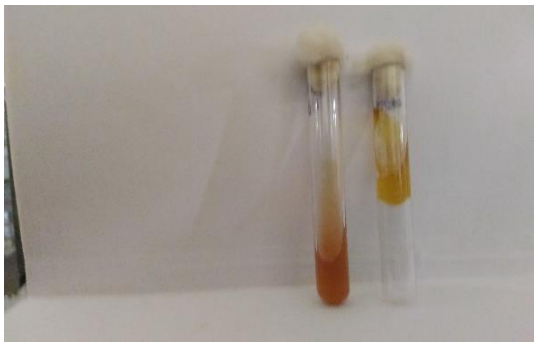

Fig. 5TSI

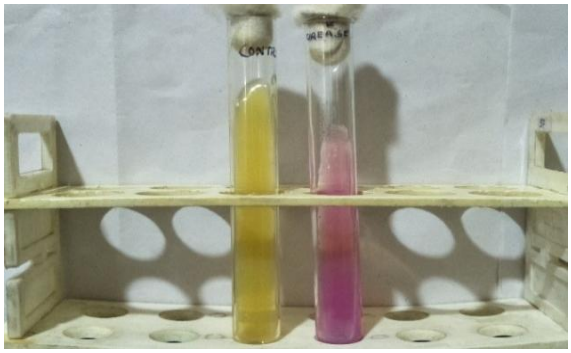

Fig. 6Urease test

3.2.2 Molecular Identification

Genomic DNA was isolated for the molecular identification and the gel image was shown in the Fig.7 PCR is also carried out for the identification of bacterium. Amplified 16s rDNA in agarose gel is shown in the Fig.8. Lane 1 represent the 1 kb DNA marker and Lane 2, 3, 6, 7, 8 is amplified 16s rDNA. A BLAST search of 16s rDNA sequence against NCBI nucleotide database revealed 100% identity with non pigmented *S. marcescens* BT47.

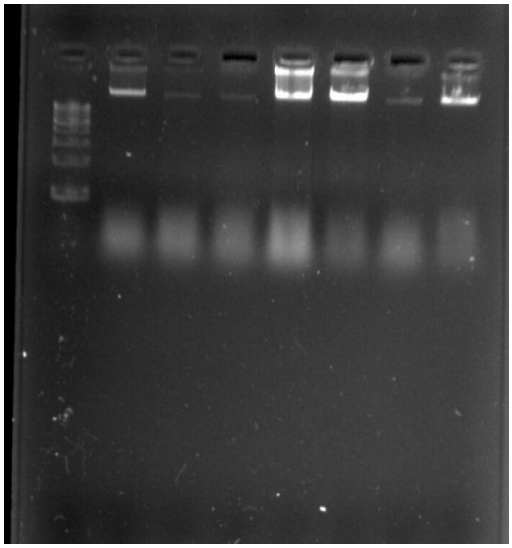

Fig.7 Genomic DNA

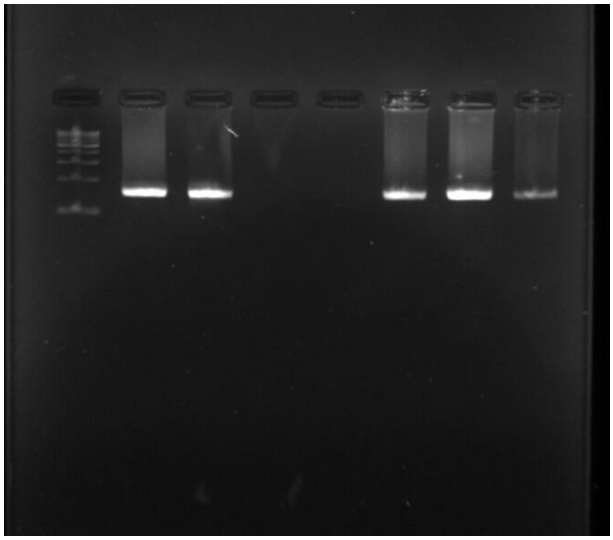

Fig.8 PCR Amplification

4.7.

3.3 Comparison of levansucrase production by SmF and SSF

Levansucrase production by *S. marcescens* BT47 by SSF was very high (104 U/ml) as compared to SmF (40 U/ml) (Fig.9)

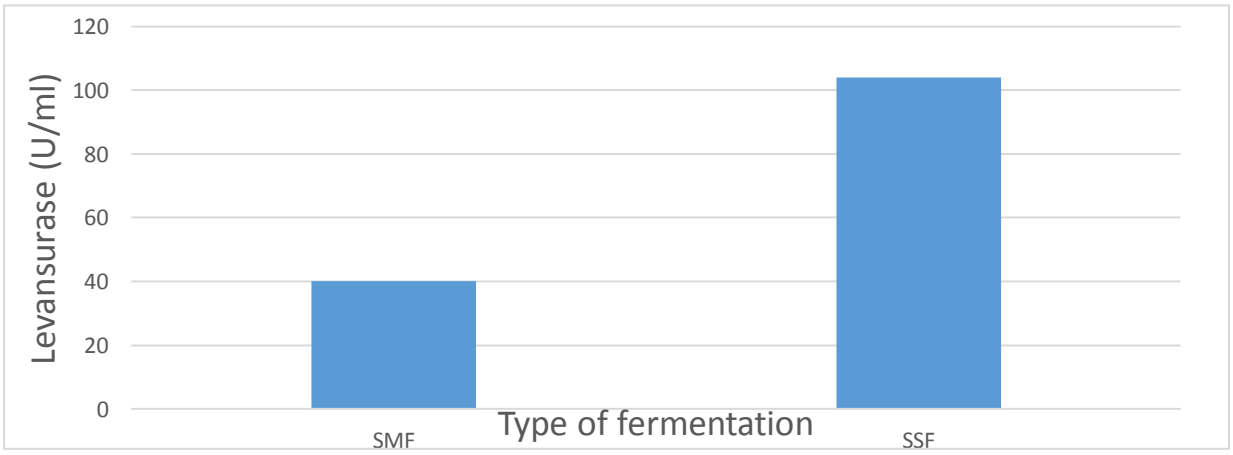

Fig.9 Comparison OF levansurase by SmF and SSF

IV. DISCUSSION

Levansucrase enzymes are becoming increasingly popular in biotechnology. Microorganisms are chosen as a source of industrial enzymes over plants and animals. Enzymes produced by microbes are more predictable and regulated, and therefore have lower manufacturing costs. According to this study, Serratia marcescens BT47, which was isolated from rotten watermelon, produced a substantial amount of levansucrase. Serratia marscescens has not been reported to produce levansucrase in the past. SSF had a productivity that was more than twice as high as SmF. Because of the potential value of levan (which is produced by the enzyme levansucrase), it is vital to find low-cost methods of generating the enzyme. Solid state fermentation, or SSF, is a low-cost alternative manufacturing technique in which the nature of the Solid Substrate (SS) is the most important factor. SS not only provide nutrients to the culture media, but they also aid in the growth of the microbial cells. Supplementation may be required if an optimum SS does not provide all of the required nutrients. SSF methods are simple, use low-volume equipment (which lowers costs), and produce high product titers (concentrated products). In this study, we used tamarind peel as an SSF substrate. Tamarind is a popular tropical fruit around the world, but the tamarind shells are discarded during the manufacturing process.

Agricultural waste is heavy and takes up a lot of landfill area, contributing to environmental issues. Not only does utilising this to make levansucrase assist the environment, but it also reduces the cost of producing levansucrase. Additional reports of successful levansucrase production are available. [14,15].

V. CONCLUSION

Serratia marcescens BT47 levansucrase production was compared under submerged fermentation (SMF) and solid state fermentation (SSF). According to our findings*S. marcescens* BT47 levansucrase production was highest under SSF utilizing tamarind shell powder as a substrate. There are no other reports of *S.marscescens* which produces levansucrase.

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