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Phylogenetic Analysis of Potential Dextran Producer *Weissella confusa*

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Abstract: Exopolysaccharide like dextran a polymer of glucose is produced by bacteria like *Leuconostoc mesenteroides*, *Lactobacillus sp*, *Weissella sp* etc. They have wide range of applications in the food, pharmaceutical and other industries. Dextran derivatives like iron dextran, clinical dextran are rapidly emerging as a new medically and industrially important product. In the present study efficient dextran producing organism was isolated from idli batter by using enrichment culture technique. It was microscopically identified by staining techniques, culturally by growth on sodium azide medium and biochemically by tests like catalase, resistance to vancomycin. The isolate was genetically identified by 16s RNA sequencing. For sequencing the sample was sent to Macrogen Korea. Sequence data was processed using bioinformatics programme like Blast N (Basic Local Alignment Search Tool) sequence alignment and neighbour joining tree construction methods. The sequenced and aligned data was used in constructing the Phylogenetic tree, which illustrate the relationship of different organisms with the help of MEGA 4.1 software (Molecular Evolutionary Genetics Analysis). The isolate was genetically identified as *Weissella confusa* and was designated as *Weissella confusa* 518 strain and it was used for dextran production using sucrose rich medium.

Keywords: Bioinformatics, Blast N, Mega 4.1, Phylogenetic tree, 16s RNA sequencing, *Weissella confusa*

I. INTRODUCTION

Microbial exopolysaccharides have great advantages over traditionally used polysaccharides derived from plants and seaweeds because these are easily influenced by environmental factors [1], and so can have much diverse applications. Dextran is one such microbial exopolysaccharide made up of glucose molecules joined into chains of varying length [2]. It is produced as low molecular weight and dextrans and high molecular weight dextrans (From 10 to 150 Kilo Daltons) [3]. Dextran is produced by microorganisms that belong to different genera like *Leuconostoc*, *Streptococcus*, *Lactobacillus*, *Gluconobacter* [4]. Mostly Leuconostoc mesenteroides was commercially used for industrial production of dextran [5]. Dextran is of particular interest because of its use as blood plasma volume expander [6]. It finds various other industrial applications in food, pharmaceutical, and chemical industries as adjuvant, emulsifier, carrier and stabilizer [7]. Dextran can serve as a potential prebiotic and can be used as food supplement for health benefits [8]. Crossed linked dextran known as sephadex are widely used for separation and purification of various products like protein in research and industry. In food industry it is being used as thickener for jams and ice cream as it prevents crystallization of sugar, improves moisture retention and maintains flavor and appearance of the food stuffs [9]. Dextran also finds applications as stabilizing and reducing agent in nanotechnology for development of silver nanoparticles by green synthesis mode [10]. Taking this fact into consideration the current study was initiated with an interest in isolating efficient dextran producing organism from idli batter using enrichment culture technique and identification of isolate by microscopically, culturally, biochemically, genetically by 16s rRNA sequencing and Phylogenetic tree was constructed using bioinformatics programme.

II. MATERIALS AND METHODS

A. Isolation of Dextran Producer

Bacterial culture was isolated from idli batter using enrichment culture technique. Sample was inoculated into a Cortezi medium [11] containing sucrose as a main carbon source and screened by using Mc Clesky medium containing 0.05% sodium azide [12].

B. Microscopic, Cultural and Biochemical Characterization

Microscopic features of dextran producer was studied by Gram staining technique, capsular staining and by spore staining. Dextran isolate was inoculated onto cortezi agar. Cortezi agar plates were incubated at 35^{o}C for 24 hours. Colony characters like color, shape, size, margin and consistency were observed [13]. Indole, methylred, Voges-Proskauer and citrate test (IMViC) were performed using Tryptone broth, glucose phosphate peptone broth and Simmons citrate agar. Catalase and oxidase test (para-aminodimethylaniline) were studied by using hydrogen peroxide and disc method [14]. The antibiotic test was performed using commercially available antibiotic disc vancomycin [15].

C. Phylogenetic Studies of Selected Isolate

Selected dextran producer was sent to Macrogen, Korea for 16s-rRNA sequencing. The 16s-rRNA sequence of the isolate was used to carry out BLAST N (Basic Local Alignment Search Tool) with the data base of National Center for Biotechnology Information GenBank database. Based on maximum identity, few sequences were selected and aligned using multiple alignment software ClustalW2 and

Phylogenetic tree was constructed using Molecular Evolutionary Genetics Analysis (MEGA 4.1) software [16].

III. RESULTS

Efficient dextran producer was isolated by enrichment culture technique with sucrose in Cortezi medium. The microscopic observation of efficient dextran producer was Gram positive, rod shaped which was both non capsulated and non sporing. Colonies on Cortezi medium were pale colored, smooth, entire and highly mucoid. The biochemical tests indicated only positive for methyl red among IMViC tests, catalase and oxidase negative and resistance to vancomycin "Table-1". For further identification the strain was sent to Macrogen, Korea and it was genetically identified by 16s-rRNA sequencing and designated as *Weissella confusa* 518 "Fig-1". A bioinformatics tool Blast N with database of NCBI GenBank, was used for more 16s-rRNA gene sequence investigation of other closely related strains. The sequence of 16s-rRNA of *Weissella confusa* 518 was 97% similar to 16s-rRNA genes from other related strains. Phylogenetic tree was constructed using Blast analysis and neighbouring joining tree construction method by using MEGA 4.1 software [16] "Fig-2".

Table-1: Characteristics of *Weissella sp*

Characteristics	*Weissella sp*
Gram staining	Gram positive, rod shaped.
Capsular staining	Non-capsulated
Spore staining	Non sporing
Colony morphology	
On Cortezi medium	Pale colored, smooth, slimy, entire.
Mc Cleskey medium	Pale colored, smooth, highly mucoid.
Biochemical tests	
Indole	-
Methyl red	+
Voges-Proskauer	-
Citrate	-
Catalase	-
Oxidase	-
Vancomycin	Resistance

>*Weissella_confusa*_dextran_culture_518
TCCGATTTTTGGGCGTGAAGACGTAGCGCAG
ACGCGTTATTTAAGTCTGAAGTGAAAGCCCTC
AGCTCAACTGAGGAATTGCTTTGGAAACTGG
ATGACTTGAGTGCAGTATAGGAAAATGGAAC
TCCATGTGTAGCGGTGAAATGCGTAGATATAT
GGAAGAACACCAGTGGCGAAGGCGGCTTTCT
GGACTGTAACTGACGTTGAGGCTCGAAAGTG
TGGGTAGCAAACAGGATTAGATACCCTGGTA
GTCCACACCGTAAACGATGAGTGCTAGGTGTT
TGAGGGTTTCCGCCCTTAAGTGCCGCAGCTAA
CGCATTAAGCACTCCGCCTGGGGAGTACGAC
CGCAAGGTTGAAACTCAAAGGAATTGACGGG
GACCCGCACAAGCGGTGGAGCATGTGGTTTA
ATTCGAAGCAACGCGAAGAACCTTACCAGGT
CTTGACATCCCTTGACAACTCCAGAGATGGAG
TGTTCCCTTCGGGGACAAGGTGACAGGTGGT
GCATGGTTGTCGTCAGCTCGTGTCGTGAGATG
TTGGGTTAAGTCCCGCAACGAGCGCAACCCTT
ATTACTAGTTGCCAGCATTCAGTTGGGCACTC
TAGTGAGACTGCCGGTGACAAACCGGAGGAA
GGTGGGGATGACGTCAAATCATCATGCCCCTT
ATGACCTGGGCTACACACGTGCTACAATGGC
GTATACAACGAGTTGCCAACCCGCGAGGGTG
AGCTAATCTCTTAAAGTACGTCTCAGTTCGGA
TTGTAGGCTGCAACTCGCCTACATGAAGTCGG
AATCGCTAGTAATCGCGGATCACACGCCGGG
GGGAAAAAACCTTTCCCCGGGGTCTTTGTACA
CCCCCGCCCGTCACACCAATGAAAAGTTTGTA
ACACCCAAAAGCCCGGTGGGGGTAACCTTCC
GGGAGCCAGCCCGTCTAAAGGTGGGACAGAT
GATTAAGGGTGATCTAGGAGGAGGACCCGCC
AAAAAAAAAAAAGGGGA

Fig.ure-1: 16s-rRNA sequence of *Weissella confusa* 518

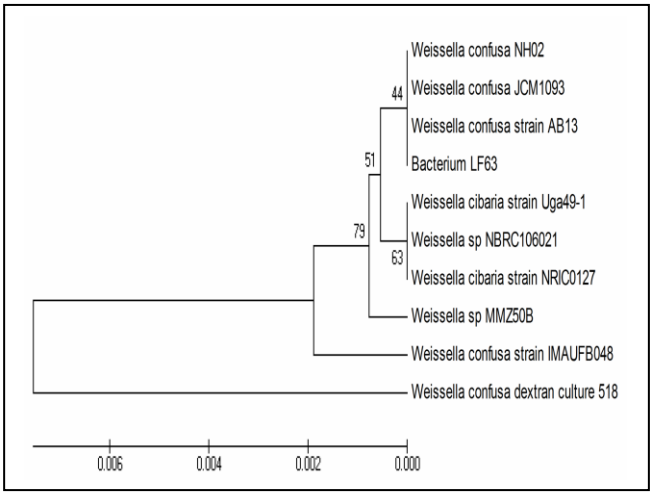

Figure-2: Phylogenetic tree of dextran producer *Weissella confusa* 518

IV. DISCUSSION

As dextran has diverse applications in food, pharmaceuticals and other industries there is not only greater demand for it, but it is also has become an integral component of different industries. There is demand for an efficient dextran producer, presently as the amount of dextran produced is practically insufficient to meet the dextran requirements of different industries. Hence, the microbial production of dextran at industrial level requires a highly productive strain. Efficient dextran producer was screened by enrichment culture technique using Cortezi medium with sucrose and screened by using Mc Clesky medium. Identification of a natural isolate is important if it is has to be commercially exploited and so the present isolate from idli batter was identified by microscopically, culturally, biochemically and genetically by 16s-rRNA sequencing. For 16s-rRNA sequencing the isolate was sent to Macrogen, Korea as these studies requires sophisticated infrastructural facilities. The isolate was identified as *Weissella confusa* and designated as *Weissella confusa* 518. The sequence of 16s-rRNA of *Weissella confusa* 518 was 97% similar to 16s-rRNA genes from other related strains. Phylogenetic tree was constructed using Blast analysis and neighbouring joining tree construction method by using MEGA 4.1 software which illustrates the relationship of different organisms.

V. CONCLUSION

An efficient strain was isolated that produced more amount of dextran in sucrose rich medium. The isolate was morphologically, biochemically and genetically by 16s-rRNA sequencing identified and designated as *Weissella confusa* 518. The isolate is Gram positive, rod shaped, non sporing and non capsulated. Colonies were highly mucoid on sucrose medium. Catalase and oxidase negative. Among IMViC reactions the isolate was positive only to methyl red. The sequence of 16s-rRNA of *Weissella confusa* 518 was 97% similar to 16s-rRNA genes of other related strains namely *Weissella confusa* AB13, *Weissella confusa* NH02, and *Weissella confusa* JCM1093.

VI. ACKNOWLEDGMENT

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VII. REFREENCES

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