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16S RNA SEQUENCING AND PHYLOGENETIC ANALYSIS OF POTENTIAL PECTINOLYTIC BACILLUS SP ISOLATED FROM VEGETABLE WASTE DUMP YARD SOILS

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Abstract: Pectinases have potential application in food industry and hence are one of the fast upcoming enzymes of commercial sector with 25% share in global market. Pectinases are of major importance in clarification of concentrated fruit juices and so are extensively used in processing of fruits and vegetables. Diverse pectin rich sources and soils were screened for pectinolytic isolates. An efficient bacterial isolate was isolated from vegetable dump yard soils showed highest polygalacturonase activity. It was identified culturally, morphologically and biochemically as Bacillus sp. The selected isolate Bacillus Sp was subjected to16s RNA sequencing and phylogenetic analysis for genetic identification. The sequenced sample data was analyzed using Bioinformatics tools like Basic Local Alignment search tool (BLAST N) for nucleotide matching and phylogenetic tree was constructed using MEGA 4.1 software (Molecular Evolution Genetic Analysis).16S gene sequencing and Phylogenetic analysis of potential pectinolytic Bacterial sp showed 96% sequence similarity with Bacillus subtilis and was labelled as Bacillus subtilisKANP. The isolate under study was further studied for pectinases and other enzymes production as these enzymes have ample applications in fruit juice industry.

Keywords: 16s RNA sequencing, Phylogenetic analysis, Polygalacturonase, Bacillus Sp KANP

I. INTRODUCTION

Pectinases are among the first enzymes to be used in industries, from as early as 1930s. Today there are two major categories of pectic enzymes i.e. acidic pectinases and alkaline pectinases that dissolve the extracellular matrix of plant tissues [1]. Pectinolytic enzymes though have a major role in fruit based food industries they have other minor applications also. It has been reported that microbial pectinases account for 25% of the global food enzymes sale and at present, majority of these commercial preparations are done from microorganisms [2]. Pectinases are the industrially important enzymes and have potential biotechnological applications in paper and pulp industries [3], textile industries [4], bio-scouring of cotton fibers [5], tea and coffee fermentation [6], oil extraction [7], waste management [5], degumming of plant bast fibers [8], retting of plant fibers [9], protoplast fusion technology and other industries. Today 75% of the estimated sale of industrial enzyme is contributed by pectinases [1]. Commercial exploitation of pectinases mainly polygalacturonases has been well established in fruit juice industry for clarification of various fruit juices. Enzymatic treatment of fruit juices for juice clarification may be done by viscometric studies [10]. In the present study polygalacturonase producer Bacillus sp isolated from vegetable dump yard soils is identified and characterized culturally, morphologically and biochemically. The bacterial strain selected is not only an efficient pectinase producer but also a multi enzyme producer. Such strains are of commercial significance in industry. Therefore it is further identified based on 16s RNA sequencing and phylogenetic analysis.

II. MATERIALS AND METHODS

A. Screening and isolation of Pectinolytic bacterial isolate

By screening different pectin rich sources and soil samples, an efficient pectinolytic bacterial isolate identified as Bacillus sp1 was isolated from vegetable waste dump yard soil [11].

B. Identification of Bacterial Isolate

An efficient highest Polygalacturonase producing bacterial isolate was identified based on cultural, morphological characteristics by growing on Czapek agar plates enriched with pectin and microscopic observation by Gram's Staining. Colony morphology was observed after 24 hours of incubation. Sugar fermentation was tested using sugars like glucose, fructose, lactose and sucrose [12].

C. 16s RNA sequencing and Phylogenetic analysis of Selected Isolate

Selected potential pectinolytic bacterial isolate was subjected to16s-rRNA sequencing (Macrogen, Korea). The sequenced sample data was analyzed using Bioinformatics tools like Basic Local Alignment search tool (BLAST N) and with the Genbank data base information of National Center for Biotechnology Information (NCBI). Nucleotide matching and phylogenetic tree was constructed using MEGA 4.1 software (Molecular Evolution Genetic Analysis) [13].

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III. RESULTS

An efficient highest Polygalacturonase producing bacterial sp was isolated from vegetable waste dump yard soil by enrichment culture technique with Czapek agar plates enriched with pectin. Bacterial isolate was identified as Bacillus sp1 by cultural, morphological and biochemical characteristics. Microscopic observation by Gram's staining and spore staining clearly indicate that the bacterial isolate as Gram positive rod shaped bacteria with sporulation. White, irregular colonies were observed on Czapek agar plates enriched with pectin. Biochemical characteristics include Indole positive, Methy red test positive, Vogues Prauskers and Citrate utilisation test positive. Gelatin liquifaction and starch hydrolysis is found be positive. Enzymatic test like Oxidase, Catalase were found to be positive and Urease negative. Fermenting abilty sugars like glucose, fructose, lactose and sucrose was found to be positive "Table- 1". Selected potential pectinolytic bacterial isolate was identified as Bacillus Sp by 16s-rRNA sequencing (Macrogen, Korea) "Fig-1". The sequenced sample data was phylogenetically analyzed using Bioinformatics tools like Basic Local Alignment search tool (BLAST N) for nucleotide matching and phylogenetic tree was constructed using MEGA 4.1 software (Molecular Evolution Genetic Analysis) [16].16S gene sequencing and Phylogenetic analysis of potential pectinolytic Bacterial sp showed 96% sequence similarity with Bacillus subtilis and was labelled as Bacillus subtilisKANP"Fig-2"

Table: 1 Cultural, microscopic and biochemical characteristics of selected pectinolytic Bacillus sp 1

Tests	Results
Colony Morphology	White, irregular colonies
Gram's Reaction	Gram positive rods
Spores staing	Sporulating
Indole	-
Methyl red	+
Vogues Prauskers	+
Citrate Utilisation	+
Starch hydrolysis	+
Gelatin liquifaction	+
Catalase	+
Urease	-
oxidase	-
Fermentation tests	
Glucose	+
Fructose	+
Lactose	+
Sucrose	+

>150406-39_C19_Culture_4-B-13-Bacillus_sp_907R.ab1 923
CCGTCCGTTCTCCCAGGCGGAGTGCTTAA
TGCGTTAGCTGCAGCACTAAG
GGGCGGAAACCCCCTAACACTTAGCACT
CATCGTTTACGGCGTGGACTAC
CAGGGTATCTAATCCTGTTCGCTCCCCAC
GCTTTCGCTCCTCAGCGTCAG
TTACAGACCAGAGAGTCGCCTTCGCCACT
GGTGTTCCTCCACATCTCTAC
GCATTTCACCGCTACACGTGGAATTCCAC
TCTCCTCTTCTGCACTCAAGT
TCCCCAGTTTCCAATGACCCTCCCCGGTT
GAGCCGGGGGCTTTCACATCA
GACTTAAGAAACCGCCTGCGAGCCCTTTA
CGCCCAATAATTCCGGACAAC
GCTTGCCACCTACGTATTACCGCGGCTGC
TGGCACGTAGTTAGCCGTGGC
TTTCTGGTTAGGTACCGTCAAGGTACCGC
CCTATTCGAACGGTACTTGTT
CTTCCCTAACAACAGAGCTTTACGATCCG
AAAACCTTCATCACTCACGCG
GCGTTGCTCCGTCAGACTTTCGTCCATTG
CGGAAGATTCCCTACTGCTGC
CTCCCGTAGGAGTCTGGGCCGTGTCTCAG
TCCCAGTGTGGCCGATCACCC
TCTCAGGTCGGCTACGCATCGTTGCCTTG
GTGAGCCGTTACCTCACCAAC
TAGCTAATGCGCCGCGGGTCCATCTGTAA
GTGGTAGCCGAAGCCACCTTT
TATGTTTGAACCATGCGGTTCAAACAACC
ATCCGGTATTAGCCCCGGTTT
CCCGGAGTTATCCCAGTCTTACAGGCAGG
TTACCCACGTGTTACTCACCC
GTCCGCCGCTAACATCAGGGAGCAAGCT
CCCATCTGTCCGCTCGACTTGC
ATGTATTAGGCACGCCGCCAGCGTCGTCT
GACGAAAAAAAAAAAAATATA
TATATAAAAAACCCCCCAAACTT

Figure-1: 16sRNA sequence of *Bacillus subtilis*

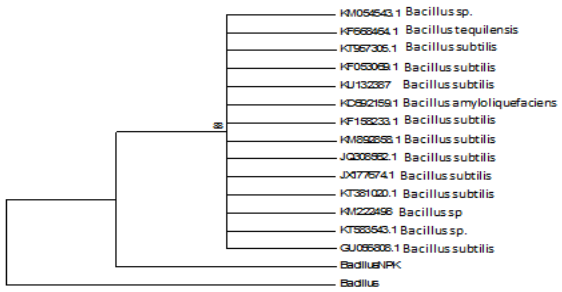

Figure-2: Phylogenetic tree of *Bacillus subtilis* KANP.

DISCUSSION

Pectinases have diverse commercial applications. They are one of the most important groups of enzymes used in fruit and vegetable industry for increasing juice yield and juice clarification. Other important applications of pectinases include in textile industries, plant fiber processing, oil extraction, waste water treatment, purification of viruses, paper making, paper and pulp industries etc. They have also been widely used in the industrial processing of wine, tea and coffee fermentations [2].

As pectinases have wide application in food and fruit juice industry there is not only greater demand for them, but also they have become an integral component of fruit juice industry. Keeping in view their diverse biotechnological applications, there is always a demand and requirement for an efficient pectinase enzyme producer with wide range of applications. Presently, the microbial synthesis of enzymes at industrial level requires highly productive strains to reduce production costs.

Considering the increasing demand of pectinases for industrial processes, the present study was initiated with an interest in isolating an efficient pectinolytic organism from diverse sources. Screening was concentrated on isolation of bacterial polygalacturonase producer with multienzyme production capacity. Isolation of pectinolytic organisms was done from various pectin rich sources. Pectins are predominantly present in both fruits and vegetables and so such pectin rich sources were screened for pectinolytic microorganisms. Up to 10% of soil samples have been reported to show pectinolytic isolates [14]. Efficient pectinase producers were screened from selected pectin rich sources. Pectinolytic bacterial strains were earlier isolated from soils and vegetable sources [15]. Diverse sources for pectinolytic isolates in nature are peels or rinds of fruits, rotten vegetables and their dump yards [16]; [17]. So similar such sources were used for initial screening in the present study. Pectinolytic isolate Bacillus sp. showed multi enzyme and also highest polygalacturonase activity. Identification of a natural isolate is important if it has to be commercially exploited and so the present isolate from vegetable dump yard soils of Hyderabad was isolated and identified as Bacillus sp. The identification of bacterial isolate to the species level was concluded based on cultural, morphological, biochemical characteristics. 16 S gene sequencing and Phylogenetic analysis revealed that the nucleotide sequence similarity of selected bacillus isolate showed maximum sequence similarity with that of the Bacillus subtilis nucleotide sequence. 16S gene sequencing and Phylogenetic analysis of potential pectinolytic Bacterial sp showed genetic similarity with Bacillus subtilis and was taxonomically labelled as *Bacillus subtilis*KANP.

CONCLUSION

An efficient bacterial isolate was isolated from vegetable dump yard soils showed highest polygalacturonase activity. It was identified culturally, morphologically and biochemically as Bacillus sp. 16S gene sequencing and Phylogenetic analysis of potential pectinolytic Bacterial sp showed 96% sequence similarity with *Bacillus subtilis* and was labelled as *Bacillus subtilis*KANP. This isolate could be used for polygalacturonase and other enzymes production as these enzymes have ample applications in fruit juice industry.

IV. ACKNOWLEDGMENT

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