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"Biochemical Characterization of Actinobacteria Isolated from Guava Orchard Soil: A Promising Source of Industrial Enzymes for Bioethanol Production"

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Abstract:

Fruit orchards are productive ecosystems where actinobacteria, particularly Streptomyces, play a key role in the degradation of lignocellulosic materials. These bacteria produce enzymes such as cellulase, xylanase, and amylase, which have significant industrial applications, including in bioethanol production. In this study, we focused on isolating and characterizing cellulolytic and xylanolytic actinomycetes from the soil of a guava (Psidium guajava) orchard in Muzaffarpur, India. Out of 30 actinomycete isolates, five best strains (SP101–SP105) were isolated and subjected to biochemical and molecular characterization using 16S rRNA sequencing. The biochemical and physiological characterization of strains SP101–SP105 revealed their ability to grow within a temperature range of 16 to 45°C, with optimal growth observed between 30 and 35°C, indicating their mesophilic nature. None of the strains (SP101–SP105) exhibited growth at temperatures of 50°C or higher. The strains also demonstrated tolerance to NaCl concentrations of up to 7%, but no growth was detected for strains SP101–SP105 at 12% NaCl or above. These findings suggest the adaptability of strains SP101–SP105 to moderate saline environments and their potential for application in bioethanol production.

Keywords : Actinobacteria, Streptomyces, Cellulase, Xylanase, Guava orchard, Bioethanol production, Lignocellulose degradation.

Introduction:

The increasing demand for renewable energy sources has intensified the need for bioethanol, a sustainable biofuel produced from lignocellulosic biomass (Abdel *et al.,* 2013; Zhao, *et al.,* 2012a; Zhao *et al.,* 2012b; Tutzenberger *et al.,* 1970 and Zhu *et al.,* 2008). Lignocellulose, the primary structural component of plant cell walls, is a complex matrix of cellulose, hemicellulose, and lignin. Microbial enzymes, particularly cellulases and xylanases, are crucial for the conversion of this biomass into fermentable sugars, which are subsequently used for bioethanol production (Gupta *et al.,* 2012; Hankin *et al.,* 1977; Lynd *et al.,* 2002; Sannigrahi *et al.,* 2010 and Shallom *et al.,* 2003).

Actinobacteria, particularly those belonging to the genus Streptomyces are prolific producers of these

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enzymes (Chater *et al.,* 1997; Deswal *et al.,* 2012 ; Ghose *et al.,* 1987; Singh *et al.,* 2012). These bacteria play a vital role in the decomposition of organic matter in soil ecosystems, including fruit orchards where large quantities of plant material are available. Despite their known significance, few studies have focused on the isolation and characterization of actinomycetes from guava orchards. This study aimed to isolate and characterize potent cellulolytic and xylanolytic actinomycetes from the soil of a guava orchard in Muzaffarpur, India, with a focus on their potential application in bioethanol production.

Materials and Methods:

Sample Collection:

Soil samples were collected from the rhizosphere of guava (Psidium guajava) plants in a fruit orchard located in Muzaffarpur, India. Using sterile tools, soil was collected from the top 15 cm layer and stored in sterile plastic bags for transport to the laboratory, ensuring minimal contamination.

Isolation of Actinobacteria:

The soil samples were air-dried, sieved, and serial dilutions were prepared for plating on ISP2 medium, supplemented with antifungal agents to inhibit fungal growth. After incubation at 30°C

Enzymatic Screening:

Isolates were screened for cellulolytic, xylanolytic, and amylolytic activities using carboxymethyl cellulose (CMC), xylan, and starch agar plates, respectively. The plates were incubated at 30°C for 48 hours, followed by flooding with specific staining reagents: Congo red for cellulase and xylanase activity, and iodine for amylase activity. Zones of clearance around colonies indicated enzyme production for 7–10 days, colonies with typical actinomycete morphology were isolated and purified on fresh ISP2 medium. Among eight media tested, ISP2 and ISP3 showed the highest colony count and morphological diversity, while ISP5 and ISP6 also performed well. In contrast, SOC, Czapek-Dox, 2xYT and R2A media yielded fewer actinomycete-like colonies. A total of 50 putative actinobacterial isolates were obtained, with strains SP101 to SP105 exhibiting significant enzymatic activity by degrading substrates like aesculin, pectin, and xylan, but not egg yolk.

Biochemical Characterization:

Five isolates (SP101–SP105) with the highest enzymatic activity were selected for further biochemical characterization. Tests performed included citrate utilization, indole production, methyl red, Voges-Proskauer, catalase, urease, H, S production, and hydrolysis of starch, casein, and gelatin.

Growth Condition Optimization:

The growth of isolates SP101–SP105 was assessed under varying NaCl concentrations (4%, 8%, and 12%) and temperatures (16°C, 30°C, 35°C, 45°C, 50°C) to determine their adaptability to different environmental conditions, which is crucial for potential industrial applications.

Results:

Isolation and Enzymatic Activity:

A total of 28 actinomycete-like colonies were isolated from the guava orchard soil. Of these, five strains (SP101–SP105) exhibited strong enzymatic

activity, with clear zones of hydrolysis observed on CMC, xylan, and starch plates. The highest cellulolytic and xylanolytic activities were observed in strains SP102 and SP104.

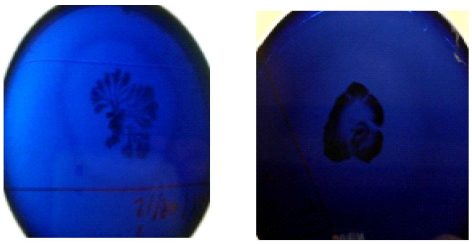

Figure 1a and 1b

Zone of Hydrolysis for cellulose degradation on modified ISP Medium No. 2 by using substrate CMC (0.5%) as model compound 1% Trypan blue and 15g/l agar by different isolated actinomycete strains SP102 and SP104 after 14 days at 30^0C.

Biochemical Characterization:

The biochemical tests revealed diverse metabolic activities among the isolates (Table 1). All strains (SP101–SP105) were positive for catalase and cellulase production, with SP101 and SP104 showing indole production. Strain SP102 demonstrated strong amylase activity, while SP105 exhibited significant casein hydrolysis. All strains grew in a temperature range of 16–45°C, with optimal growth at 30–35°C, classifying them as mesophiles. Moderate halotolerance was observed, with growth at 4% and 7% NaCl, but none of the strains grew at 12% NaCl or higher.

Effect of NaCl on Isolated Strains:

The effect of varying NaCl concentrations on the growth of the isolated strains is summarized in Table 2. All strains showed good growth at 4% and 8%

Table 1.: Biochemical Characterization of Isolated Actinobacterial Strains

Sl. No.	Tests	Bacterial Strains*				
		SP101	SP102	SP103	SP104	SP105
1	Citrate test	+	+	+	+	+
2	Indole Test	+	+	+	+	+
3	Methyl Red test	+	-	-	-	-
4	VP test	-	-	-	-	-
5	Catalase test	+	+	+	+	+
6	Decarboxylase Test	+	+	+	+	+
7	Urease Test	-	-	+	+	+
8	H_2S Test	-	-	+	+	+
9	Starch hydrolysis	-	+	+	+	+
10	Cellulose hydrolysis	+	+	+	+	+

NaCl, but no growth was observed at NaCl concentrations of 12% or higher.

Table 2. Effect of NaCl Concentration on the Growth of Isolated Strains:

Effect of NaCl concentration	SP101	SP102	SP103	SP104	SP105
4%	+	+	+	+	+
8%	+	+	+	+	+
12%	-	-	-	-	-
16%	-	-	-	-	-

Effect of Temperature on Isolated Strains:

The isolated strains showed growth over a temperature range of 16°C to 45°C, with optimal growth observed at 30°C and 35°C. No strains were able to grow at temperatures above 50°C (Table 3).

Molecular Identification:

16S rRNA sequencing confirmed the identity of the isolates as members of the genus Streptomyces. The isolates shared 98-99% similarity with known cellulolytic Streptomyces species in the GenBank database, confirming their potential in lignocellulose degradation.

Growth Condition Optimizatio:

All five isolates grew optimally at 30°C to 35°C and tolerated NaCl concentrations up to 8%. No growth was observed at NaCl concentrations above 12% or at temperatures above 45°C, indicating that the isolates are mesophilic and moderately halotolerant.

Discussion:

The results of this study underscore the potential of actinomycetes, particularly Streptomyces species, isolated from guava orchard soils for industrial applications. The cellulolytic and xylanolytic activities observed in strains SP101–SP105 are of particular interest for bioethanol production, as these enzymes are crucial for breaking down lignocellulosic biomass into fermentable sugars. The ability of these strains to grow in varying NaCl concentrations and temperatures suggests that they could be adapted for use in industrial bioconversion processes under different environmental conditions. Strains SP102 and SP104, which exhibited the highest cellulolytic and xylanolytic activities, are prime candidates for further investigation, particularly in the context of bioethanol production from lignocellulosic biomass. The identification of these strains as members of the genus Streptomyces further validates their potential, as Streptomyces species are known for producing industrially relevant enzymes.

Conclusion:

The isolation of actinomycetes from guava orchard soil in Muzaffarpur, India, yielded five strains with strong cellulolytic and xylanolytic activities, indicating their potential for bioethanol production. The adaptability of these strains to different environmental conditions further enhances their industrial applicability. Future studies should focus on optimizing enzyme production and investigating the genetic basis of lignocellulose degradation in these strains.

Limitations:

This study was limited to a single guava orchard and sample size was relatively small. Further studies are required to explore the enzymatic potential of

Table 3. Temperature Growth Range of Isolated Strains:

Temperature growth range (°C)	SP101	SP102	SP103	SP104	SP105
16°	+	+	+	+	+
30°	+++	+++	+++	+++	+++
35°	+++	+++	+++	+++	+++
45°	+	++	++	+	++
50°	-	-	-	-	-
55°	-	-	-	-	-
65°	-	-	-	-	-

actinomycetes from other fruit orchards and to assess the scalability of enzyme production for industrial use.

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