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Feather degradation by actinobacteria: molecular characterization and biotechnological applications

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Abstract

This study focused on the isolation and characterization of keratinase-producing actinobacteria from feather dumping soil. Based on the clearance zones formed on the medium, selected colonies were subjected to secondary screening using a modified basal liquid medium supplemented with raw chicken feathers. Out of ten isolates, only three - JJCPDKTA2, JJCPDKTA3, and JJCPDKTA9 - were further examined for their morphological, physiological, and biochemical characteristics. In addition, 16S rRNA sequencing and phylogenetic analysis identified them as *Streptomyces variabilis*, *Streptomyces azureus*, and a member of *Actinomycetales*. These isolates were deposited in the GenBank database and assigned accession numbers: *S. variabilis* (KR909307), *S. azureus* (KR909308), and *Actinomycetales* sp. (KR909309). The findings demonstrate the potential of these actinobacteria in keratinase production, highlighting their efficiency in feather degradation through keratinolytic activity.

Keywords: Actinobacteria, Feather degrading soil, 16S rRNA analysis

Introduction

Actinomycetes are widely distributed in nature and play a crucial role in the degradation of organic matter. Soil serves as a natural reservoir for these microorganisms and their antimicrobial metabolites, making it an excellent source for the discovery of therapeutically valuable compounds [1].

Keratin waste is generated in large amounts across many countries. Despite being rich in proteins and carbon compounds, it often remains underutilized, and improper disposal contributes to environmental pollution [2]. Poultry-processing industries, in particular, generate millions of tons of feathers annually as waste by-products [3].

Actinomycetes, one of the most prevalent groups of Gram-positive, primarily aerobic filamentous bacteria, are well recognized for their metabolic diversity, enabling survival under extreme environmental conditions. Many actinomycetes are of ecological and industrial importance, being used in the production of antibiotics and enzymes [4]. Keratin, abundant in amino acids such as leucine and serine, can be efficiently degraded by keratinase enzymes, most of which are extracellular and inducible by keratin waste [5].

Materials and methods

Study area

Soil samples were collected from the feather waste dumping site in Pudukkottai district, Tamil Nadu, India. The district covers an area of 4,663 km² with a coastline of 42 km. Samples were obtained at a depth of 30 cm from the feather disposal site, placed in sterile bags, and transported to the laboratory for immediate processing (Figure 1).

Soil physico-chemical analysis

The soil sample was analyzed for organic carbon, total nitrogen, phosphorus, potassium, sodium, calcium, magnesium, sulfur, chloride, zinc, iron, manganese, boron, and molybdenum content. Analyses were performed by the Government of Tamil Nadu Department of Agriculture, Trichy (Table 1).

Isolation and screening of actinobacteria

Suspected actinobacterial isolates were maintained on ISP1 medium. Primary screening for keratinolytic activity was performed on milk agar plates, and the formation of clear zones was taken as evidence of activity [6].

Phenotypic characterization

Spore surface morphology was examined using a scanning electron microscope after 14 days of incubation [8]. Growth was tested under varying sodium chloride concentrations (5–12%), temperatures (15–42 °C), and pH values (6–9) using ISP1 medium.

Molecular characterization

Genomic DNA was extracted from actinobacterial cells grown in yeast malt extract broth supplemented with 0.2% glycine [10]. DNA amplification was carried out using PCR, followed by purification of the amplified fragments via gel electrophoresis.

Sequencing was performed with a Taq DyeDeoxy terminator cycle sequencing kit (Applied Biosystems) and standard oligonucleotide primers.

The 16S rRNA sequences were aligned with reference sequences from GenBank/EMBL/DDBJ databases using CLUSTAL W (version 1.81). Phylogenetic analysis was conducted using the neighbor-joining method [11].

Results

Isolation and screening

Ten isolates (JJCPDKTA1–JJCPDKTA10) were recovered from actinobacterial isolation agar. Primary screening on milk agar plates revealed keratinolytic activity in three isolates: JJCPDKTA2, JJCPDKTA3, and JJCPDKTA9. These isolates were further subjected to secondary screening for feather degradation, showing complete degradation of chicken feathers in modified basal liquid medium within 15–25 days (Figures 2–3).

Morphological and cultural characteristics

The isolates exhibited good growth on ISP1 medium, producing dried, powdery white colonies with visible substrate mycelium (Table 2). On ISP media ISP1–ISP7, they demonstrated excellent growth and abundant aerial mycelium formation (Figures 4–6). Optimal growth occurred at 28 °C and pH 7–8, with reduced growth at both lower and higher pH values (Table 3).

Molecular Characterization

The 16S rRNA genes of isolates JJCPDKTA2, JJCPDKTA3, and JJCPDKTA9 were partially sequenced. Analysis revealed high sequence similarity (95–100%) to *Streptomyces variabilis*, *Streptomyces azureus*, and other members of Actinomycetales. BLAST (N) searches against GenBank, EMBL, and DDBJ confirmed up to 98% sequence homology. Sequences were deposited in GenBank, and accession numbers were obtained (Figures 4, 6, 8–9).

Discussion

Colony characteristics, aerial mycelium pigmentation, and spore surface morphology are critical parameters for the identification of *Streptomyces* species [8]. In this study, isolates showed variation in spore surface structures, ranging from smooth to warty, consistent with earlier reports [10,15].

While physiological tests provide useful markers for recognizing individual strains, they are often insufficient for species-level identification [12]. Molecular approaches, particularly sequencing of ribosomal RNA genes (16S, 23S, and 5S), remain the most reliable tools for actinobacterial taxonomy. In particular, 16S rRNA gene sequencing offers valuable insights into phylogenetic placement and evolutionary relationships [14].

The feather-degrading isolates obtained in this study not only demonstrate strong keratinolytic activity but also highlight the potential of actinobacteria in biotechnological applications, including waste management and enzyme production.

Table 1: Physico – chemical analysis of the feather dumping soil sample

S. No	Name of The Parameter	Sample
1.	Organic Carbon (%)	2.49
2.	Total Nitrogen (%)	1.21
3.	Total Phosphorus (%)	0.50
4.	Total Potassium (%)	3.02
5.	Total Sodium (%)	0.05
6.	Total Calcium (%)	3.89
7.	Total Magnesium (%)	3.06
8.	Total Sulfur (%)	0.52
9.	Total Chloride (%)	1.68
10.	Total Zinc (ppm)	2.09
11.	Total Copper (ppm)	0.40
12.	Total Iron (ppm)	85.19
13.	Total Manganese (ppm)	50.56
14.	Total boron (ppm)	0.61
15.	Total Molybdenum (ppm)	0.02

Table 2: Morphological Characterization of 3 isolates

Medium	JJCpdktA2			JJCpdktA3			JJCpdktA9		
	Growth Pattern	Aerial mycelium	Reverse pigmentation	Growth pattern	Aerial mycelium	Reverse pigmentation	Growth pattern	Aerial mycelium	Reverse pigmentation
ISP1 Tryptone Yeast Agar	Poor	-	-	Good	Milky white	Pale yellow	Good	Pale yellow	Pale white
ISP2 Yeast Meal Agar	Poor	-	-	Good	Milky white	Pale yellow	Good	Pale white	Pale yellow
ISP3 Oat Meal Agar	Good	white	White	Good	white	Pale white	Good	Gray	Gray
ISP4 Inorganic Salt Starch Agar	Good	white	Gray	Good	Gray	Pale white	Good	Pale yellow	white
ISP5 Glycerol Asparagine Agar	Good	Pale white	White	Good	white	Pale yellow	Good	Pale white	White
ISP6 Peptone Yeast Extract Iron	Poor	-	-	Good	Milky white	Yellow	Poor	-	-
ISP7 Tyrosine Agar Base	Poor	-	-	Good	Milky white	Pale yellow	Poor	-	-

Table 3: Physiological and bio-chemical characterization of actinobacteria

Characteristics	*Streptomyces Variabilis*	*Streptomyces Azureus*	*Actinomycetales*
Spore chain morphology	*Rectiflexibiles*	Spirals	Chains
Spore surface	*No data available*	Smooth	No data available

Aerial mass color	*White*	Pale white	Cremy white
Soluble pigment	*Yellow*	None to faintly tinged with brown	None
Carbon source			
D-Glucose	+	+	+
L-Arabinose	+	+	_
D-Xylose	+	+	+
D-Fructose	+	+	+
D-Mannitol	+	+	+
Inositol	_	+	+
Sucrose	_	+	+

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