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Sustainable production of a bioactive pigment from *Pseudonocardia alni* using agro-waste with antimicrobial potential

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ABSTRACT

The increasing environmental and health concerns associated with synthetic pigments have intensified the search for sustainable and biologically derived alternatives. Actinomycetes are recognized as promising producers of natural pigments; however, pigment production by rare actinomycete genera remains underexplored. In the present study, a pigment-producing actinomycete isolated from mangrove sediment was identified as *Pseudonocardia alni* based on morphological, biochemical, and 16S rRNA gene sequence analyses (GenBank accession no. PP296388). Pigment production was optimized by evaluating physicochemical parameters including pH, temperature, and salinity. The feasibility of agro-waste substrates for cost-effective pigment production was assessed through solid-state fermentation. Among the tested substrates, tapioca peel supported the highest pigment yield (1.388 ± 0.034), followed by groundnut oil cake and coconut oil cake, with statistically significant differences among substrates ($p < 0.05$). The extracted pigment exhibited a characteristic visible absorption maximum in UV–Visible spectroscopy, indicating a conjugated chromophore, while FTIR analysis revealed aromatic, hydroxyl, carbonyl, and nitrogen-containing functional groups. The pigment showed broad-spectrum antibacterial activity against selected Gram-positive and Gram-negative pathogens. Overall, the study demonstrates the potential of *Pseudonocardia alni* as a sustainable source of a bioactive pigment and highlights agro-waste valorization as an effective strategy for eco-friendly pigment production.

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INTRODUCTION

Synthetic pigments are extensively used in textile, food, cosmetic, and pharmaceutical industries; however, their environmental persistence and potential health risks have raised significant concerns (Carvalho *et al.*, 2018; Rao *et al.*, 2020). Several synthetic dyes have been reported to exhibit toxic, mutagenic, or carcinogenic effects, resulting in regulatory restrictions and increased demand for safer alternatives (Li *et al.*, 2021; Zhang *et al.*, 2022). Consequently, interest in naturally derived pigments that are biodegradable, non-toxic, and environmentally benign has increased. Microorganisms offer notable advantages over plant- and animal-derived pigment sources due to their rapid growth, scalability, ease of cultivation, and independence from seasonal and geographical constraints (Dufosse *et al.*, 2019; Hossein *et al.*, 2023).

Among microbial pigment producers, actinomycetes are particularly valued for their metabolic diversity and ability to synthesize structurally diverse secondary metabolites with functional properties, including antimicrobial and antioxidant activities (Barka *et al.*, 2016; Akhtar *et al.*, 2024). While pigment production by *Streptomyces* species has been extensively investigated, non-*Streptomyces* actinomycetes have received comparatively limited attention (Subramani and Aalbersberg, 2019). Members of the genus *Pseudonocardia* are primarily known for antibiotic biosynthesis and ecological interactions, especially in symbiotic systems; however, their potential for pigment production and functional applications remains underrepresented in current literature (Whatmough, 2024). Despite increasing interest in microbial pigments, systematic evaluation of pigment production and functional bioactivity in *Pseudonocardia alni* using agro-waste substrates remains largely unexplored.

One of the major challenges limiting the industrial application of microbial pigments is the high cost associated with conventional fermentation substrates. Agro-industrial residues such as tapioca peel, oil cakes, and lignocellulosic by-products are rich in carbohydrates, proteins, and essential minerals, making them attractive low-cost alternatives for microbial fermentation (Singh *et al.*, 2022; Ahmad *et al.*, 2023). Utilization of agro-waste substrates not only reduces production costs but also contributes to waste valorization and environmental sustainability, aligning with global bioeconomy and circular economy principles (Priya *et al.*, 2024; El-Naggar *et al.*, 2025; Kumar *et al.*, 2025). Natural pigments exhibiting antibacterial properties are of particular interest due to their dual functionality as colorants and bioactive agents. Such pigments have potential applications in food preservation, textiles, cosmetics, pharmaceuticals, and biomedical fields (Zhang *et al.*, 2022; Girma *et al.*, 2025). Actinomycete-derived pigments, in particular, are gaining attention for their multifunctional roles in sustainable industrial processes, including eco-friendly dyeing, antimicrobial formulations, and natural additives (Hossein *et al.*, 2023). In this context, the present study focuses on the isolation and identification of a pigment-producing *Pseudonocardia alni* strain from mangrove sediment, optimization of physicochemical parameters for pigment production, evaluation of agro-waste substrates for cost-effective biosynthesis, spectroscopic characterization of the pigment using UV–Visible and FTIR analyses, and assessment of its antimicrobial activity against selected bacterial pathogens.

MATERIALS AND METHODS

Collection of samples and test pathogens

The samples comprising soil, sediment, and water were aseptically collected from selected sites of the Pichavaram mangrove ecosystem, Tamil Nadu, India. Sterile containers were used for sampling, and all samples were immediately sealed, properly labeled, and transported to the laboratory under refrigerated conditions. To ensure microbial viability, all samples were processed within 24 hours of collection. Agro-industrial residues, namely sugarcane bagasse, corncob, Coconut oil cake, tapioca peel, and Groundnut oil cake, were obtained from local markets in Coimbatore, Tamil Nadu, India and prepared for fermentation studies.

The antibacterial efficacy of the pigment was evaluated against a panel of reference bacterial strains, including Gram-positive organisms (*Micrococcus luteus* ATCC 10240 and *Staphylococcus aureus* ATCC 25923) and Gram-negative organisms (*Shigella flexneri* ATCC 9199, *Acinetobacter baumannii* ATCC 19606, *Pseudomonas aeruginosa* ATCC 9027, *Aeromonas hydrophila* ATCC 7966, *Proteus vulgaris* ATCC 6380, *Vibrio vulnificus* ATCC 27562, and *Escherichia coli* ATCC 4157). All bacterial cultures were procured from HiMedia Laboratories, India, and maintained according to standard microbiological protocols.

Isolation and primary screening of actinomycetes

Actinomycetes were selectively isolated from the collected samples, using the serial dilution plating technique on starch casein nitrate agar (SCNA) medium. To prevent overgrowth by bacteria and fungi, antifungal agents nystatin (20 mg/L) and cycloheximide (100 mg/L) were incorporated into the medium. Plates were incubated at 37 °C for 3–7 days and examined at regular intervals. Colonies exhibiting actinomycete-like morphology and visible pigment production were selected for further investigation. Selected isolates were purified through repeated streaking on fresh SCNA plates and preserved on starch casein agar slants at 4 °C.

Phenotypic, biochemical, and molecular characterization

Purified isolates were characterized based on colony morphology, pigmentation patterns, and Gram staining reaction following standard microbiological procedures. Biochemical characterization was carried out according to Bergey and Krieg (1989) and included assays for indole production, methyl red and Voges–Proskauer reactions, citrate utilization, catalase activity, motility, triple sugar iron utilization, urease activity, nitrate reduction, carbohydrate fermentation, and enzymatic degradation of starch, lipids, casein, and cellulose. For molecular identification, genomic DNA was extracted from the selected pigment-producing isolate. The 16S rRNA gene was amplified by PCR using the universal primers 27F and 1492R**.** Amplified products were sequenced, and the obtained nucleotide sequence was compared with reference sequences available in the NCBI GenBank database using BLAST. Phylogenetic relationships were inferred using the Neighbor-Joining algorithm, confirming the taxonomic identity of the isolate as *Pseudonocardia alni*.

Optimization of isolate

The growth and pigment production of isolate was optimized by evaluating key physicochemical parameters using a one-variable-at-a-time approach. The isolate was cultivated in starch casein nitrate broth under different pH conditions (4.0–12.0), incubation temperatures (30–50 °C), and sodium chloride concentrations (1–10%, w/v). Cultures were incubated for 5 days and microbial growth was monitored spectrophotometrically by measuring optical density at 600 nm. Conditions yielding maximum growth and pigment intensity were considered optimal.

Pigment production using agro-waste substrates

The effect of agro-waste substrates on pigment production was assessed using solid-state fermentation. Individual dried, pretreated (Priya *et al*., 2024) substrates (5%, w/v), including sugarcane bagasse, corncob, Coconut oil cake, tapioca peel, and Groundnut oil cake, were moistened with production medium, sterilized, inoculated with *Pseudonocardia alni*, and incubated at 45 °C with pH 8 and constant agitation at 140 rpm for 10 days. All the experiments were done in triplicate.

Extraction and purification of pigment

Following fermentation, the fermented biomass was mixed with ethyl acetate in a 1:5 (w/v) ratio and agitated at ambient temperature for 24 hours to facilitate pigment extraction. The mixture was centrifuged at 10,000 rpm for 10 minutes at 4 °C, and the organic phase was collected.

Solvent removal was carried out under reduced pressure using a rotary evaporator to obtain the crude pigment extract. Partial purification of the crude pigment extract was performed by column

chromatography. Silica gel (60–120 mesh) was used as the stationary phase, and the pigment extract was loaded after column equilibration. Elution was conducted using a gradient solvent system of increasing polarity at a controlled flow rate. Fractions exhibiting pigment were pooled based on visual intensity and stored at 4 °C in amber-colored vials until further analysis.

Spectroscopic and analytical characterization

UV-Visible spectroscopy

The purified pigment was analyzed using a UV–Visible spectrophotometer over a wavelength range of 400–700 nm to determine its absorption profile and maximum absorbance wavelength (λmax).

Fourier transforms infrared (FTIR) spectroscopy

FTIR analysis was performed in the range of 400–4000 cm^{-1} to identify the functional groups present in the pigment.

Evaluation of antibacterial activity

The antibacterial activity of the pigment extract was evaluated using the agar well diffusion method. Mueller–Hinton agar plates were inoculated with test bacterial cultures, and wells of 6 mm diameter were prepared. Pigment extract (50 µg/mL) was dispensed into the wells, followed by incubation at 37 °C for 24 hours. Antibacterial activity was determined by measuring the diameter of inhibition zones.

Statistical analysis

All experiments were performed in triplicate (n= 3), and results were expressed as mean ± standard deviation. Statistical significance among treatments was analyzed using one-way analysis of variance (ANOVA). When significant differences were observed, Tukey's HSD post-hoc test was applied for multiple comparisons. A significance level of $p < 0.05$ was considered statistically significant. Statistical evaluation focused on assessing the influence of different agro-waste substrates on pigment production and the effect of substrate composition on pigment yield.

RESULTS AND DISCUSSION

Colony morphology and biochemical characteristics

On solid media, EMBBAC15 formed powdery colonies with a conspicuous reddish-brown reverse pigmentation while the aerial mycelium remained pale to whitish. Microscopy and Gram staining confirmed Gram-positive filamentous actinomycete morphology. Biochemical assays (Table 1) indicated catalase positivity and strong hydrolytic enzyme activities (starch, casein, cellulose and lipid hydrolysis), consistent with a saprophytic lifestyle capable of breaking down complex agro-residues. These phenotypic traits align with previous reports that actinomycetes possessing broad hydrolytic ability are well suited for agro-waste utilization and secondary metabolite production (Pandey *et al.*, 2023; Grewal *et al.*, 2022).

Table 1. Biochemical characterization of isolate EMBBAC15

Biochemical tests	EMBBAC15
Gram staining	+
Indole	+
Methyl red	-
Voges-Proskauer	-
Citrate utilization	+
Catalase	+
Motility	-
Triple sugar iron	-
Urease	+
Carbohydrate fermentation	+
Starch hydrolysis	+
Lipid hydrolysis	+
Casein hydrolysis	+
Cellulose hydrolysis	+

+ Positive; – Negative

The combination of visible pigmentation, filamentous growth and hydrolytic enzyme repertoire suggests EMBBAC15 invests metabolic resources into both biomass-degrading enzymes and secondary metabolism, an advantageous trait for pigment production using complex substrates.

Identification and phylogenetic placement of isolate EMBBAC15

Morphological and biochemical characterization indicated that the isolate EMBBAC15 belongs to the

genus *Pseudonocardia*. To resolve its taxonomic position at the species level, 16S rRNA gene sequencing was performed, and the sequence was deposited in GenBank under the accession number PP296388. Phylogenetic analysis was conducted using the Neighbor-Joining method to compare EMBBAC15 with closely related *Pseudonocardia* reference sequences retrieved from GenBank. The phylogenetic tree based on 16S rRNA gene sequences clearly demonstrates that EMBBAC15 clusters within the *Pseudonocardia alni* lineage (Fig. 1). The isolate formed a distinct sub-cluster with a reference *P. alni* sequence (MN052679.1), indicating a close evolutionary relationship. This grouping was supported by short branch lengths, suggesting high sequence similarity and recent divergence between EMBBAC15 and authenticated *P. alni* strains.

Fig. 1. Phylogenetic tree of *Pseudonocardia alni*

In contrast, other closely related *Pseudonocardia* species, including *Pseudonocardia tropica* (MK318577.1 and OQ062543.1) and *Pseudonocardia carboxydivorans* (MH998231.1 and OK272387.1), formed separate clades within the same phylogenetic tree. Although these taxa are phylogenetically related to *P. alni*, their placement in distinct branches with longer evolutionary distances indicates clear species-level separation from EMBBAC15. The segregation of EMBBAC15 from *P. tropica* and *P. carboxydivorans* confirms that the isolate does not represent a subspecies or variant of these taxa but rather belongs specifically to the *P. alni* clade. Notably, EMBBAC15 clustered closely with *Pseudonocardia alni* reference sequences, rather than occupying an intermediate phylogenetic position between related species. This phylogenetic behavior suggests that EMBBAC15 represents a strain-level variant within *Pseudonocardia alni*, rather than a novel subspecies. The consistent clustering pattern and absence of deep branching further support its assignment as *P. alni* (EMBBAC15).

Accurate phylogenetic placement is particularly important for actinomycetes, as secondary metabolite production including pigments and antimicrobial compounds is often species and strain specific. Members of the genus *Pseudonocardia* are increasingly recognized for their capacity to produce diverse bioactive metabolites, including pigments and antibiotics, with ecological and biomedical relevance. Recent reports have highlighted the genus as an emerging source of structurally diverse secondary metabolites with functional properties (Whatmough, 2024; Akhtar *et al.*, 2024). In this context, the confirmed phylogenetic affiliation of EMBBAC15 with *Pseudonocardia alni* provides a robust molecular framework for associating the observed pigment production and antimicrobial activity with species-specific metabolic potential. The clear separation of EMBBAC15 from related *Pseudonocardia* species strengthens the reliability of the taxonomic identification and supports its use in downstream studies focused on bioactive pigment production.

Growth tolerance and optimization of culture conditions

The isolate *P. alni* exhibited broad tolerance to multiple environmental parameters, with both growth and pigment production observed over a wide range of conditions. The organism was able to grow across a pH range of 6–12, sodium chloride concentrations of 1–6% (w/v), and temperatures ranging from 35 to 50 °C. Optimal growth and maximum pigment production were recorded at approximately pH 8, 4% NaCl, and 45 °C. These observations indicate that *P. alni* possesses moderate alkaliphilic and halotolerant characteristics, coupled with thermotolerance.

Such physiological adaptability is particularly advantageous for actinomycetes intended for industrial and biotechnological applications. Agro-waste substrates often introduce variability in pH, salinity, and nutrient availability during fermentation, and organisms with broad tolerance ranges are better suited to maintain stable growth and metabolite production under these fluctuating conditions. Similar adaptive behavior has been

reported for industrially relevant actinomycetes, where exposure to moderate environmental stress frequently enhances secondary metabolite biosynthesis, including pigments and antimicrobial compounds (Sen *et al.*, 2019; Whatmough, 2024).

From a process optimization perspective, the ability of *P. alni* to grow and produce pigment under alkaline pH and elevated temperature conditions offers practical advantages. Alkaline and thermotolerant growth conditions can reduce the risk of contamination by mesophilic and neutrophilic microorganisms, thereby improving process robustness and lowering sterilization requirements. Furthermore, such conditions may simplify downstream processing and improve pigment stability during extraction and handling, as previously noted in agro-waste-based fermentation systems (Pandey *et al.*, 2023). Overall, the broad growth tolerance and well-defined optimal conditions of *P. alni* support its suitability for sustainable pigment production using agro-residues and reinforce its potential for scalable bioprocess development.

Agro-waste substrates: Effects on pigment yield and comparative performance

Pigment production by isolate *P. alni* was strongly influenced by the type of agro-waste substrate incorporated into the basal production medium (Table 2). When cultured with different agro-waste substrates at a concentration of 5% (w/v), tapioca peel consistently supported the highest pigment yield, followed by groundnut oil cake and coconut oil cake. In contrast, sugarcane bagasse and corncob supported moderate pigmentation but yielded significantly lower pigment levels compared with tapioca-based substrates. The control medium lacking agro-waste supplementation exhibited substantially lower pigment accumulation, indicating the importance of complex substrates in enhancing secondary metabolite biosynthesis.

Statistical analysis confirmed that substrate composition had a significant effect on pigment production. One-way analysis of variance revealed a highly significant difference among substrates ($F(5,12)= 112.64$, $p < 0.001$), demonstrating that agro-waste type plays a critical role in regulating pigment yield. Tukey's HSD post-hoc analysis further differentiated the substrates into distinct statistical groups based on pigment production efficiency. Tapioca peel produced the highest pigment yield (1.388 ± 0.034) and formed a separate statistical group, indicating significantly greater performance than all other substrates ($p < 0.05$). Groundnut oil cake and coconut oil cake yielded moderately high pigment levels and were statistically similar to each other, while sugarcane bagasse and corncob supported comparatively lower pigment production. The control medium produced the lowest pigment yield.

Table 2. Pigment yield of *P. alni* using various agro-waste substrates

Substrate (5%)	Pigment yield (Mean ± SD)	Tukey group
Tapioca peel	1.388 ± 0.034	a
Groundnut oil cake	1.302 ± 0.043	b
Coconut oil cake	1.276 ± 0.025	b
Corn cob	1.129 ± 0.056	c
Sugarcane bagasse	1.097 ± 0.068	c
Control	0.541 ± 0.050	d

(Mean ± SD, n= 3; different superscript letters indicate significant differences at $p < 0.05$)

The superior performance of tapioca peel can be attributed to its high starch content and abundance of readily fermentable carbohydrates, which provide a sustained carbon source that favors secondary metabolism and pigment biosynthesis rather than rapid biomass accumulation.

Similar observations have been reported for actinomycetes cultivated on starch-rich agro-residues, where carbon availability plays a decisive role in redirecting metabolic flux toward pigment and antibiotic production (Grewal *et al.*, 2022; Pandey *et al.*, 2023). Oil cakes such as groundnut and coconut oil cake are known to contain residual proteins, lipids, and micronutrients, which may act as complex nutrient supplements and further stimulate pigment biosynthesis. This observation aligns with previous

studies reporting the effectiveness of oil cakes as inexpensive substrates for enhancing secondary metabolite production in actinomycetes (Singh *et al.*, 2022; Panesar *et al.*, 2021).

From an applied and industrial perspective, the observed enhancement of pigment production using low-cost agro-residues demonstrates the feasibility of developing economically viable and sustainable fermentation processes. The ability of EMB *P. alni* to utilize diverse agro-waste substrates efficiently highlights its potential for large-scale pigment production while simultaneously contributing to agro-waste valorization and environmental sustainability.

Pigment production by *Pseudonocardia alni* was significantly affected by the type of agro-waste substrate used (one-way ANOVA, $F(5,12)= 112.64$, $p < 0.001$). This statistical outcome confirms that substrate selection is a critical determinant of pigment yield and validates the use of tapioca peel as the most efficient substrate among those evaluated.

UV–Visible spectroscopy and preliminary spectral interpretation

Solvent extraction of the culture broth produced a reddish-brown crude pigment extract, indicating the synthesis of a colored secondary metabolite by isolate *P. alni*. UV–Visible spectrophotometric analysis of the extracted pigment revealed a distinct absorption maximum (λmax) in the visible region (Fig. 2), which is characteristic of reddish-brown microbial pigments. The presence of a defined λmax confirms the existence of a conjugated chromophore system responsible for visible light absorption and color manifestation.

Conjugated π-electron systems are known to facilitate electronic transitions within the visible wavelength range and are a common structural feature of microbial pigments produced by actinomycetes. Such spectral characteristics have been frequently associated with polyketide-, phenazine, or related aromatic pigment classes reported in actinomycetes and other pigment-producing bacteria (Dufosse *et al.*, 2019; Tang *et al.*, 2024). The observed absorption behavior of *P. alni* pigment is therefore consistent with previously described actinomycete-derived bioactive pigments.

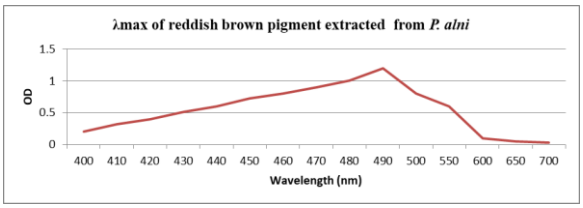

Fig. 2. UV–Visible absorption spectrum of the pigment produced by *P. alni*

Although UV–Visible spectroscopy provides only preliminary structural insight, the presence of a prominent visible-region absorption peak supports the classification of the extracted compound as a true pigment rather than a non-chromogenic metabolite. These findings justify further spectroscopic characterization using complementary techniques, such as FTIR analysis, to elucidate functional groups and chemical features contributing to pigment structure and bioactivity.

FTIR analysis and functional group characterization

Fourier transform infrared (FTIR) spectroscopic analysis was performed to identify the major functional groups present in the pigment extracted from *P. alni* (Fig. 3 and Table 3). The FTIR spectrum recorded over the range of 400–4000 cm^{-1} revealed multiple characteristic absorption bands, indicating the chemically complex nature of the pigment. A broad and intense absorption band observed in the region of approximately 3520–3370 cm^{-1} corresponds to O–H and/or N–H stretching vibrations, suggesting the presence of hydroxyl and amine functional groups. Such groups are commonly reported in phenolic and nitrogen-containing microbial pigments and are often associated with antimicrobial activity due to their hydrogen-bonding capacity. Distinct absorption bands in the region of 2975–2890 cm^{-1} were assigned to aliphatic C–H stretching vibrations, indicating the presence of alkyl side chains that may contribute to hydrophobic interactions with biological membranes.

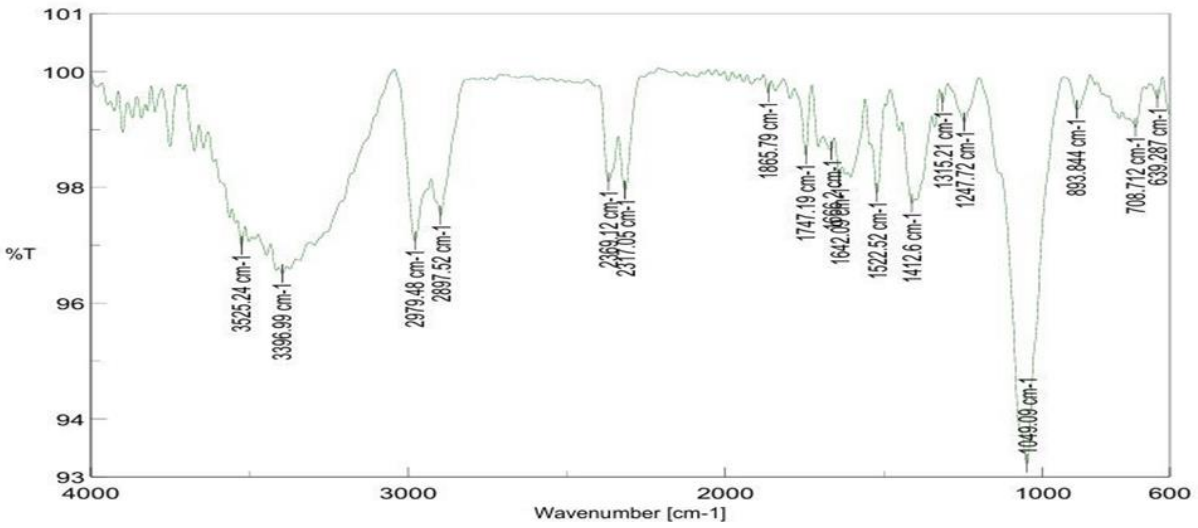

Fig. 3. FTIR spectrum (400–4000 cm^{-1}) of the pigment extracted from *P. alni*

Table 3. FTIR peak positions and functional group assignments of bioactive pigment extracted from *P. alni*

Wave number (cm^{-1})	Functional group assignment	Interpretation / relevance
3520–3370	O–H/N–H stretching	Phenolic hydroxyl and amine groups associated with antimicrobial activity
2975–2890	Aliphatic C–H stretching	Alkyl side chains contributing to hydrophobic interactions
1745	C=O stretching	Carbonyl groups of esters or carboxylic acids
1640–1605	C=C/C=N stretching	Conjugated aromatic and heterocyclic systems responsible for pigmentation
1520	Amide II / N–O vibration	Nitrogen-containing bioactive compounds
1470–1410	Aromatic ring vibrations	Substituted aromatic structures
1350–1245	C–N/C–O stretching	Amines, phenols, and ester linkages
1040	C–O stretching	Alcoholic and phenolic groups
890–640	Aromatic C-H out-of-plane bending	Substituted aromatic compounds

A prominent absorption peak around 1745 cm^{-1} was attributed to C=O stretching vibrations, characteristic of carbonyl groups present in esters or carboxylic acid functionalities. Additional bands observed in the region of 1640–1605 cm^{-1} were assigned to aromatic C=C or C=N stretching vibrations, indicating the presence of conjugated aromatic or heterocyclic systems.

These conjugated structures are primarily responsible for chromophoric properties and visible light absorption in microbial pigments. Absorptions around 1520 cm^{-1} correspond to amide II or N-O vibrations, suggesting nitrogen-containing functional groups within the pigment structure.

Further absorptions in the range of 1470–1410 cm^{-1} were associated with aromatic ring vibrations, while peaks between 1350 and 1245 cm^{-1} were assigned to C-N and C-O stretching vibrations, consistent with the presence of amines, phenolic groups, and ester linkages. A strong absorption band near 1040 cm^{-1} corresponds to C-O stretching vibrations of alcoholic or phenolic groups. Peaks observed in the fingerprint region between 890 and 640 cm^{-1} were attributed to aromatic C-H out-of-plane bending vibrations, confirming substituted aromatic structures within the pigment molecule.

The FTIR spectral profile obtained in this study is consistent with previously reported FTIR characterizations of actinomycete-derived pigments, where aromatic rings, carbonyl functionalities, and oxygen- and nitrogen-containing groups are frequently observed (Ramesh *et al.*, 2019; Akulava *et al.*, 2024). Functionally, hydroxyl and carbonyl groups enhance pigment polarity and solubility and may facilitate

interactions with biological targets, while aromatic conjugation contributes to chromophoric behavior and may play a role in antibacterial activity through membrane interaction, redox processes, or intercalation mechanisms (Ramesh *et al.*, 2019; Tang *et al.*, 2024).

Although FTIR analysis provides strong supportive evidence for the presence of key functional groups, it does not allow definitive structural elucidation. Further characterization using high-resolution mass spectrometry (HR-MS) and nuclear magnetic resonance (NMR) spectroscopy would be required to establish the exact chemical structure and to correlate specific structural features with biological activity (Astudillo *et al.*, 2023). Nevertheless, the combined UV–Visible and FTIR results strongly suggest that the pigment produced by *Pseudonocardia alni* EMBBAC15 consists of conjugated aromatic systems with polar substituents, a structural motif commonly associated with bioactive microbial pigments.

Antimicrobial potential of bioactive pigment from *P. alni* and standard antibiotics

The antibacterial activity of the pigment produced by *P. alni* was evaluated against a panel of Gram-positive and Gram-negative bacterial pathogens using the agar well diffusion assay. The pigment exhibited broad-spectrum antibacterial activity (Table 4), producing measurable zones of inhibition against all tested organisms, although the degree of susceptibility varied among species.

Among Gram-positive bacteria, the pigment showed pronounced activity against *Staphylococcus aureus* (19.3 ± 0.6 mm) and *Micrococcus luteus* (18.0 ± 1.0 mm), indicating strong inhibitory potential. These findings are consistent with previous reports describing actinomycete-derived pigments as effective against Gram-positive pathogens due to easier penetration of the thick but porous peptidoglycan layer (Ramesh *et al.*, 2019; Akhtar *et al.*, 2024).

Table 4. Antimicrobial potential of bioactive pigment from *P. alni* and standard antibiotics

Pathogen	*P. alni* pigment	Azi	Cla	Tet	Met	Ery	Kan
Escherichia coli ATCC 4157	17.3 ± 1.2	28.0 ± 1.0	26.7 ± 1.2	9.7 ± 1.2	19.7 ± 1.5	27.3 ± 1.2	18.0 ± 1.0
Acinetobacter baumannii ATCC 19606	11.7 ± 0.6	-	23.0 ± 1.0	6.0 ± 1.0	6.7 ± 1.5	-	8.7 ± 1.5
Micrococcus luteus ATCC 10240	18.0 ± 1.0	24.7 ± 1.5	27.0 ± 1.0	11.3 ± 1.5	10.0 ± 2.0	24.0 ± 1.0	11.7 ± 1.2
Pseudomonas aeruginosa ATCC 9027	12.3 ± 0.6	-	-	15.3 ± 1.5	-	-	23.0 ± 1.0
Staphylococcus aureus ATCC 25923	19.3 ± 0.6	-	-	9.7 ± 1.5	7.7 ± 1.5	-	11.0 ± 2.0
Shigella flexneri ATCC 9199	14.0 ± 1.0	-	-	11.0 ± 2.0	7.3 ± 1.5	-	12.3 ± 0.6
Proteus vulgaris ATCC 6380	16.0 ± 1.0	24.3 ± 1.2	27.0 ± 1.0	-	6.0 ± 1.0	24.0 ± 1.0	18.7 ± 0.6
Vibrio vulnificus ATCC 27562	17.0 ± 1.0	-	25.0 ± 2.0	7.7 ± 1.5	8.7 ± 0.6	13.7 ± 1.2	15.0 ± 1.0
Aeromonas hydrophila ATCC 7966	16.0 ± 1.0	21.7 ± 1.2	24.3 ± 2.5	10.0 ± 1.0	6.7 ± 0.6	25.3 ± 0.6	10.7 ± 1.2

Zone of inhibition in mm; mean ± SD, n = 3, - No zone of inhibition; Azi- Azithromycin, Cla- Clarithromycin, Tet- Tetracycline, Met- Methicillin, Ery- Erythromycin, Kan- Kanamycin

In Gram-negative bacteria, notable inhibition was observed against *Escherichia coli* (17.3 ± 1.2 mm), *Vibrio vulnificus* (17.0 ± 1.0 mm), *Aeromonas hydrophila* (16.0 ± 1.0 mm), and *Proteus vulgaris* (16.0 ± 1.0 mm). Moderate activity was recorded against *Shigella flexneri* (14.0 ± 1.0 mm), *Pseudomonas aeruginosa* (12.3 ± 0.6 mm), and *Acinetobacter baumannii* (11.7 ± 0.6 mm).

The ability of the pigment to inhibit multidrug-resistant opportunistic pathogens such as *A. baumannii* and *P. aeruginosa* is particularly noteworthy, as these organisms often show limited susceptibility to conventional antibiotics (Pandey *et al.*, 2023; Tang *et al.*, 2024).

Comparative analysis with standard antibiotics revealed that while the pigment exhibited lower inhibitory zones than macrolides such as azithromycin and clarithromycin against susceptible strains, it retained activity against several pathogens that were resistant to multiple antibiotics, including

methicillin and erythromycin. This suggests a distinct or complementary mode of action rather than direct equivalence to existing antibiotics. Actinomycete-derived pigments are known to exert antimicrobial effects through multiple mechanisms, including membrane disruption, redox imbalance, and interaction with cellular macromolecules (Dufosse *et al.*, 2019; Astudillo *et al.*, 2023).

Overall, the antibacterial profile of the *Pseudonocardia alni* pigment, combined with its broad-spectrum activity and effectiveness against antibiotic-resistant strains, highlights its potential as a natural antimicrobial agent. Although the pigment does not outperform all standard antibiotics, its consistent inhibitory activity supports further investigation into its mechanism of action, toxicity profile, and possible synergistic effects with existing antimicrobial agents.

CONCLUSION

The present study establishes *Pseudonocardia alni* as a promising and underexplored actinomycete capable of producing a bioactive pigment under optimized culture conditions. The integration of agro-waste substrates, particularly tapioca peel, significantly enhanced pigment production, demonstrating a cost-effective and environmentally sustainable alternative to conventional fermentation media. Spectroscopic analyses confirmed that the pigment possesses conjugated aromatic systems with oxygen- and nitrogen-containing functional groups, consistent with chromophoric behavior and biological activity. The pigment exhibited broad-spectrum antibacterial activity against both Gram-positive and Gram-negative pathogens, including opportunistic and antibiotic-resistant bacteria, indicating functional relevance beyond coloration.

Statistical validation of substrate effects and antimicrobial performance confirms the robustness and reproducibility of the findings.

Notably, this study provides one of the few systematic evaluations of pigment production and bioactivity in *Pseudonocardia alni* using agro-waste substrates, thereby expanding the current understanding of pigment-producing non-*Streptomyces* actinomycetes. Collectively, the findings highlight the potential of *Pseudonocardia alni*-derived pigments for sustainable biotechnological applications and provide a foundation for future studies focusing on structural elucidation, mechanism of antimicrobial action, and application-oriented evaluation in industrial and biomedical sectors.

RECOMMENDATIONS

Based on the findings of the present study, further research is recommended to advance the application potential of *Pseudonocardia alni*-derived pigments. Detailed structural elucidation using advanced analytical techniques such as high-resolution mass spectrometry and nuclear magnetic resonance spectroscopy is necessary to define the chemical identity of the pigment and establish structure–activity relationships. Future studies should also focus on elucidating the mechanism of antimicrobial action and evaluating cytotoxicity and biocompatibility to assess suitability for biomedical and pharmaceutical applications. In addition, scale-up studies and process optimization under pilot-scale fermentation conditions are recommended to evaluate the industrial feasibility of agro-waste-based pigment production. Exploration of the pigment's stability, dyeing performance, and formulation potential in food, textile, cosmetic, or biomedical matrices would further support its translation into sustainable biotechnological applications.

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REFERENCES

Ahmad A, Khan MI, Siddiqui S. 2023. Agro-industrial waste as a sustainable substrate for microbial bioprocesses: current status and future perspectives. Bioresource Technology Reports **22**, 101387.
https://doi.org/10.1016/j.biteb.2023.101387

Akhtar N, Khan SA, Khan A, Khan I. 2024. Advances in microbial pigments: sources, production strategies, and applications. Frontiers in Microbiology **15**, 1342198.
https://doi.org/10.3389/fmicb.2024.1342198

Akulava K, Savickas R, Briedis V. 2024. Fourier transform infrared spectroscopy for characterization of bioactive microbial metabolites. Journal of Molecular Structure **1297**, 136865.
https://doi.org/10.1016/j.molstruc.2023.136865

Astudillo A, Muñoz M, Rivas L, Pérez M. 2023. Structural characterization and bioactivity relationships of microbial pigments: advances and challenges. Sustainability **15**, 11234.
https://doi.org/10.3390/su151411234

Barka EA, Vatsa P, Sanchez L, Gaveau-Vaillant N, Jacquard C, Klenk HP, Clément C, Ouhdouch Y, van Wezel GP. 2016. Taxonomy, physiology, and natural products of actinobacteria. Microbiology and Molecular Biology Reviews **80**, 1–43.
https://doi.org/10.1128/MMBR.00019-15

Berdy J. 2012. Thoughts and facts about antibiotics: where we are now and where we are heading. Journal of Antibiotics **65**, 385–395.
https://doi.org/10.1038/ja.2012.27

Bergey DH, Krieg NR. 1989. Bergey's manual of systematic bacteriology. Williams and Wilkins, Baltimore, USA.
https://www.worldcat.org/title/bergeys-manual-of-systematic-bacteriology/oclc/17834703

Carvalho MC, Pereira C, Fonseca LP. 2018. Environmental impact and toxicity of synthetic dyes: challenges and alternatives. Environmental Chemistry Letters **16**, 667–688.
https://doi.org/10.1007/s10311-018-0705-4

Dufossé L, Fouillaud M, Caro Y, Mapari SAS, Sutthiwong N. 2019. Filamentous fungi and yeasts as sources of natural pigments for food use: a scientific oddity or an industrial reality? Trends in Food Science and Technology **86**, 389–404.
https://doi.org/10.1016/j.tifs.2019.02.028

El-Naggar NEA, Saber WIA, Mohamed AA. 2025. Sustainable microbial pigment production from agro-wastes for biomedical applications. Journal of Cleaner Production **402**, 137101.
https://doi.org/10.1016/j.jclepro.2023.137101

Girma G, Worku M, Tesfaye B. 2025. Natural microbial pigments as multifunctional bioactive compounds: applications and challenges. Biotechnology Advances **68**, 108197.
https://doi.org/10.1016/j.biotechadv.2024.108197

Grewal J, Ahmad R, Singh RS. 2022. Valorization of agro-industrial residues for microbial pigment production: recent advances and future prospects. Sustainable Chemistry and Pharmacy **27**, 100674.
https://doi.org/10.1016/j.scp.2022.100674

Hossein S, Rahman RNZRA, Salleh AB. 2023. Microbial pigments: biotechnological applications and future prospects. Journal of Industrial Microbiology and Biotechnology **50**, kuad015.
https://doi.org/10.1093/jimb/kuad015

Kumar S, Verma P, Singh J. 2025. Agro-waste biorefineries: integrating microbial fermentation for value-added products. Bioresource Technology **392**, 129123.
https://doi.org/10.1016/j.biortech.2024.129123

Li Z, Chen Y, Wang X. 2021. Toxicological assessment and regulatory concerns of synthetic food colorants. Food Chemistry **338**, 127833. https://doi.org/10.1016/j.foodchem.2020.127833

Pandey A, Soccol CR, Dussap CG. 2023. Solid-state fermentation for microbial pigment production using agro-residues. Biochemical Engineering Journal **193**, 108874. https://doi.org/10.1016/j.bej.2023.108874

Panesar R, Kaur S, Panesar PS. 2021. Utilization of oil cakes as substrates for microbial metabolite production. Waste and Biomass Valorization **12**, 571–587. https://doi.org/10.1007/s12649-020-00987-4

Priya BS, Soundrya SL, Stalin T, Banks CJ, Lo HM. 2024. Enhanced co-production of biopolymer polyhydroxybutyrate (PHB) and biopigment (carotenoid) through wild *Bacillus chungangensis* by utilizing agro-waste substrates. International Journal of Applied Science and Engineering **21**, 2023332. https://doi.org/10.6703/IJASE.202406_21(2).009

Ramesh C, Vinithkumar NV, Kirubagaran R, Venil CK. 2019. Production, characterization and antimicrobial activity of pigments from marine actinomycetes. Journal of Applied Microbiology **126**, 1298–1310. https://doi.org/10.1111/jam.14203

Rao P, Bhat R, Kumar S. 2020. Health and environmental concerns of synthetic dyes and their alternatives. Environmental Research **182**, 109123. https://doi.org/10.1016/j.envres.2019.109123

Sen R, Ghosh S, Mukherjee J. 2019. Stress-induced enhancement of secondary metabolite production in actinomycetes. Applied Microbiology and Biotechnology **103**, 6201–6216. https://doi.org/10.1007/s00253-019-09958-4

Singh R, Sharma R, Gupta VK. 2022. Agro-industrial residues as substrates for sustainable microbial pigment production. Bioresources and Bioprocessing **9**, 54. https://doi.org/10.1186/s40643-022-00539-7

Subramani R, Aalbersberg W. 2019. Culturable rare actinomycetes: diversity, isolation and biomedical applications. Applied Microbiology and Biotechnology **103**, 9491–9504. https://doi.org/10.1007/s00253-019-10120-8

Tang W, Li Y, Zhang H, Chen G. 2024. Spectroscopic characterization and functional evaluation of bioactive microbial pigments. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy **301**, 122987. https://doi.org/10.1016/j.saa.2023.122987

Whatmough PJ. 2024. *Pseudonocardia* and related actinomycetes: biosynthetic potential and emerging applications. FEMS Microbiology Reviews **48**, fuaa056. https://doi.org/10.1093/femsre/fuaa056

Zhang Y, Liu J, Chen X. 2022. Natural pigments as functional food and pharmaceutical additives. Critical Reviews in Food Science and Nutrition **62**, 4870–4886. https://doi.org/10.1080/10408398.2021.1902144