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DIVERSITY OF SOIL ACTINOMYCETES FROM DIVERSE HABITATS OF SOLAPUR–RAICHUR REGION AND OPTIMIZATION OF FERMENTATION PARAMETERS FOR BIOCONVERSION OF COMPACTIN TO PRAVASTATIN

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ABSTRACT
Actinomycetes remain one of the most prolific sources of clinically and industrially important secondary metabolites, yet their diversity in many Indian agro-industrial soils is still incompletely documented. In this study, soil samples were collected from 14 distinct habitats across the Solapur–Raichur belt of Maharashtra and Karnataka, India — including industrial, agricultural, riverine and compost-associated sites — and screened for actinomycete diversity using a combination of selective isolation media, antibiotic supplementation, and physical/chemical pretreatment strategies. Yeast extract–malt extract–dextrose agar supplemented with cycloheximide (25 µg/mL), fluconazole (50 µg/mL), nystatin (25 µg/mL) and rifampicin (50 µg/mL) gave contamination-free recovery and was selected for the study. Among pretreatments, a combination of dry heat (40 °C), 0.5% calcium carbonate and 0.5% phenol yielded the highest isolate recovery. A total of 308 morphologically distinct actinomycete isolates were recovered, dominated by white, grey, black and creamish aerial-mycelium morphotypes, with black and brown soils yielding the greatest isolate density. All 308 isolates were screened by thin-layer chromatography for their ability to bioconvert compactin to pravastatin via whole-cell hydroxylation; isolates G4 and G7 (from Sangvi village soil, Akkalkot) showed the strongest reaction intensity. Fermentation parameters were subsequently optimized for these two isolates: starch–casein–dextrose broth (SCDM), pH 7.0–7.5 and 28 °C gave the best bioconversion activity. These findings identify G4 and G7 as promising candidates for further scale-up, enzyme characterization and molecular identification as industrial biocatalysts for pravastatin production.

KEYWORDS: Actinomycetes; soil diversity; selective isolation; pretreatment; compactin; pravastatin; bioconversion; fermentation optimization

1. INTRODUCTION

Actinomycetes, a group of Gram-positive, filamentous, spore-forming bacteria belonging to the phylum Actinobacteria, are recognized as one of the richest sources of bioactive secondary metabolites of pharmaceutical and industrial significance. Actinomycetes are widely distributed across terrestrial and extreme habitats and continue to be a goldmine for the discovery of novel antibiotics, enzyme inhibitors, antitumor agents and other pharmacologically active compounds [1]. Soil remains the principal reservoir of culturable actinomycetes, and their distribution and diversity are strongly influenced by soil type, pH, organic content, and anthropogenic activity in the surrounding habitat.

Selective isolation of actinomycetes from soil is complicated by the overwhelming abundance of fast-growing bacteria and fungi that outcompete slower-growing actinomycete colonies on non-selective media. Foundational work by Hayakawa and co-workers established that physical pretreatments (dry heat, air-drying, centrifugation) and chemical pretreatments (phenol, calcium carbonate, antibiotics incorporated into the isolation medium) markedly improve the selective recovery of actinomycetes, including rare genera, from soil [2–5]. These principles continue to underpin selective isolation protocols used across diverse habitats, from agricultural soils to karstic caves [6].

Among the pharmaceutically important bioconversions carried out by actinomycetes, the microbial hydroxylation of compactin (mevastatin, ML-236B) to pravastatin — a clinically important HMG-CoA reductase inhibitor used for the management of hypercholesterolemia — is of particular industrial interest. Chemical synthesis of pravastatin from compactin is costly and generates unwanted stereoisomeric by-products, making microbial biotransformation the preferred industrial route [7]. Streptomyces carbophilus, Actinomadura species and related actinomycetes have been reported to carry out the 6β-hydroxylation of compactin to pravastatin via inducible cytochrome P450 systems or constitutive hydroxylases [7–10]. Continued bioprospecting for novel, high-yielding actinomycete strains from unexplored soil habitats therefore remains directly relevant to the pharmaceutical biotechnology industry.

The present study was undertaken with the objectives of (i) isolating and characterizing culturable actinomycetes from 14 diverse soil habitats of the Solapur–Raichur region, (ii) optimizing the isolation medium, antibiotic supplementation and pretreatment strategy for maximal, contamination-free recovery, (iii) screening the resulting isolate collection for compactin-to-pravastatin bioconversion activity by thin-layer chromatography, and (iv) optimizing fermentation medium, pH and temperature for the most active isolates identified.

2. MATERIALS AND METHODS

2.1 Soil sample collection

Soil samples were collected from 14 locations spanning industrial, agricultural, riverine, and processing-industry habitats across the Solapur (Maharashtra) and Raichur (Karnataka) region, India (Table 1). Three replicate samples were collected per location. Samples were transported in sterile containers, air-dried in a laminar air-flow cabinet, and stored at 2–8 °C until processing.

Code	Site / habitat	Soil description
A	NTPC area, Solapur	Ash-containing soil
B	Lingusugur–Raichur (non-agricultural)	Mixture of black and brown soil
C	Compost pond, Solapur	Light brown compost soil
D	Nasik Road agricultural land	Dark black, water-holding, fertile soil
E	Sugarcane industry, Hotgi, Solapur	Black soil with rock particles
F	Venkeys Oil Industry, MIDC, Solapur	Oil-containing soil
G	Sangvi village, Akkalkot–Solapur	Black and wet soil
H	Cow barn area, Solapur	Black and brown soil mixture
I	Shahabad tile/rock mining area	Soil with rock particles
J	Grape garden soil, Jat–Sangli	Black fertile soil
K	Nandini Milk Industry, Raichur	Soil with rock particles
L	Jurala Dam vicinity, Raichur	Black, water-holding soil
M	Fertilizer industry area, Nasik	Soil with rock particles
N	Krishna riverbank, Raichur	Sandy soil

Table 1. Soil sample collection sites and habitat description.

2.2 Selective isolation medium and antibiotic optimization

Four candidate media — yeast extract–malt extract–dextrose agar (YMA), starch casein agar, glycerol asparagine agar, and oatmeal agar — were compared for actinomycete recovery, each initially supplemented with cycloheximide (25 µg/mL), fluconazole (20 µg/mL), nystatin (25 µg/mL) and rifampicin (25 µg/mL). Serial dilutions of an air-dried representative black soil sample were plated in quintuplicate on each medium. Colonies were confirmed as actinomycetes by dry colony morphology and filamentous aerial mycelium under light microscopy. Following medium selection, antibiotic concentrations were further optimized on YMA using black soil sample A, comparing combinations of cycloheximide, fluconazole, nystatin and rifampicin at concentrations from 20–50 µg/mL to minimize bacterial and fungal contamination while preserving actinomycete recovery.

2.3 Physical and chemical pretreatment of soil

Soil sample A was used to evaluate pretreatment strategies on the optimized YMA-antibiotic medium (cycloheximide 25 µg/mL, fluconazole 50 µg/mL, nystatin 25 µg/mL, rifampicin 50 µg/mL). Physical pretreatments tested were dry heat (40, 50, 60 and 70 °C for 30 min) and centrifugation (4000 rpm for 5 and 10 min). Chemical pretreatments tested were phenol (0.5–2.0% v/v) and calcium carbonate (0.5–2.0% w/v). A combined pretreatment (dry heat 40 °C + calcium carbonate 0.5% + phenol 0.5%) was also evaluated, following established precedents for physicochemical enrichment of actinomycetes from soil [2,6].

2.4 Isolation across all soil samples and morphological characterization

The selected medium, antibiotic combination and pretreatment protocol were applied to all 41 soil samples collected from the 14 locations. Isolates were assigned codes corresponding to their site of origin (A1, A2 … N23, etc.) and characterized for aerial mycelium pigmentation. Confirmation as actinomycetes was based on dry, powdery colony morphology, Gram-positive staining, and observation of branching filamentous mycelium, spores and hyphae under light microscopy.

2.5 Seed culture and submerged fermentation

Seed cultures were prepared by suspending slant growth in 5 mL sterile saline and inoculating 200 µL into 25 mL seed medium (soyabean meal 10.0 g/L, corn steep liquor 1.0 g/L, dextrose 10 g/L, soluble starch 1.0 g/L, calcium carbonate 2 g/L) in 250 mL conical flasks, incubated at 28 °C, 220 rpm for 72 h. For production fermentation, 2.5 mL of 48 h seed culture was inoculated into 25 mL production medium (soyabean meal 20.0 g/L, corn steep liquor 2.0 g/L, dextrose 20 g/L, soluble starch 20.0 g/L, calcium carbonate 2 g/L, pH adjusted to 7.20 before sterilization) and incubated at 28 °C, 220 rpm for 216 h in duplicate flasks.

2.6 Screening for compactin-to-pravastatin bioconversion

All 308 isolates were screened for their ability to hydroxylate compactin to pravastatin using lysed whole-cell preparations in the presence of NADPH and molecular oxygen. Reaction products were resolved by thin-layer chromatography using chloroform:methanol:acetic acid (85:15:1, v/v/v) as the mobile phase, and spots were visualized under UV light. Reaction intensity was scored qualitatively on a four-point scale (+, ++, +++, ++++) based on spot intensity relative to a compactin standard.

2.7 Fermentation parameter optimization

For the most active isolates identified by screening (G4 and G7), five candidate fermentation media (starch–casein–dextrose broth [SCDM], soybean–sucrose–starch–yeast extract medium, ISP medium, CSP-YE complex medium, and M9 basal medium) were compared for bioconversion activity. Using the best-performing medium, pH (6.0–8.0) and temperature (25–32 °C) were subsequently optimized, with bioconversion activity assessed qualitatively by TLC spot intensity as described above.

3. RESULTS

3.1 Selection of isolation medium

Yeast extract–malt extract–dextrose agar gave the highest recovery of actinomycetes (13 isolates), followed by starch casein agar and glycerol asparagine agar (10 isolates each) and oatmeal agar (7 isolates). YMA was therefore selected as the isolation medium for the full study.

3.2 Antibiotic concentration optimization

In the absence of antibiotics, heavy bacterial and fungal contamination was observed across all plates. Progressive optimization identified cycloheximide 25 µg/mL, fluconazole 50 µg/mL, nystatin 25 µg/mL and rifampicin 50 µg/mL as the combination giving the highest isolate recovery (13 isolates) with no visible contamination (Table 2).

Antibiotic combination	Isolates recovered	Observation
No antibiotics	—	Heavy contamination in every plate
Cycloheximide 50, Fluconazole 50, Nystatin 50, Rifampicin 50 µg/mL	6	No contamination; slow isolate growth
Cycloheximide 25, Fluconazole 50, Nystatin 25, Rifampicin 25 µg/mL	10	No contamination
Cycloheximide 25, Fluconazole 50, Nystatin 25, Rifampicin 50 µg/mL	13	No contamination — selected combination

Table 2. Optimized antibiotic supplementation of the isolation medium.

3.3 Effect of physical and chemical pretreatment

Dry heat pretreatment at 40 °C for 30 min gave the highest isolate recovery (14 isolates) among the temperatures tested; recovery declined progressively at higher temperatures, with no isolates recovered at 70 °C. Centrifugation at 4000 rpm for 10 min modestly improved recovery over the untreated control. Phenol at 0.5–1.0% and calcium carbonate at 0.5% each independently enhanced isolate recovery over untreated soil. The combined pretreatment of dry heat (40 °C) with 0.5% calcium carbonate and 0.5% phenol gave the highest overall recovery (16 isolates from soil sample A), confirming that physical and chemical enrichment act complementarily rather than redundantly (Figure 3).

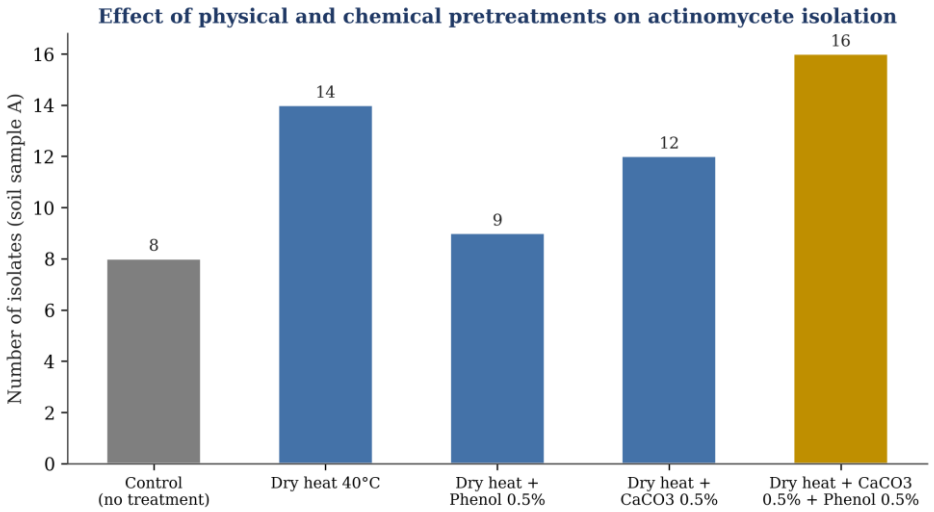

Figure 1. Effect of physical and chemical pretreatments on actinomycete isolate recovery from soil sample A.

3.4 Diversity and distribution of isolates

Applying the optimized isolation protocol to all 41 samples from 14 locations yielded 308 actinomycete isolates. Isolate density varied substantially by site, ranging from 15 isolates (site F, oil industry soil) to 34 isolates (site G, Sangvi village soil). Black and brown soils generally gave the highest isolate densities, consistent with reports that actinomycete abundance correlates with organic-matter-rich, moderately alkaline soils (Table 3, Figure 1).

Code	Isolates	Code	Isolates	Code	Isolates
A	18	F	15	K	20
B	26	G	34	L	18
C	22	H	23	M	16
D	22	I	28	N	23
E	26	J	17	Total	308

Table 3. Distribution of the 308 actinomycete isolates across the 14 soil sample sites.

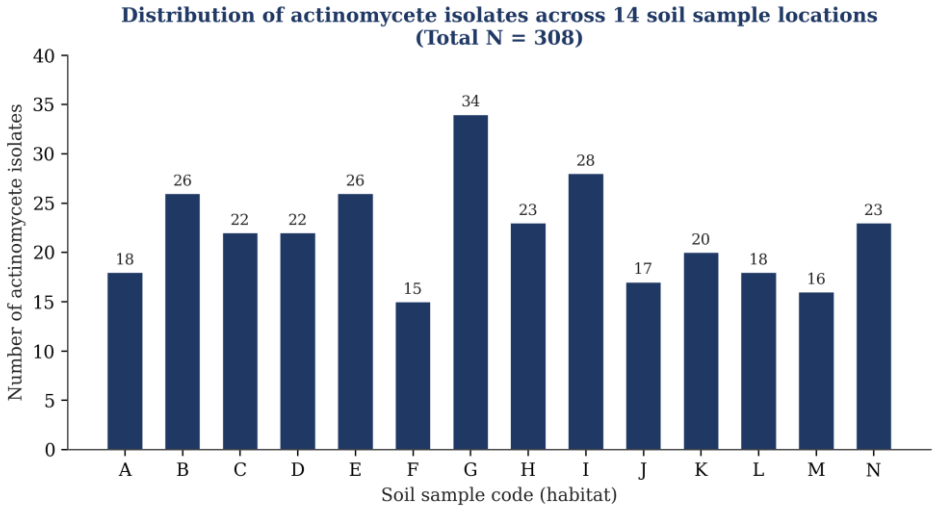

Figure 2. Distribution of actinomycete isolates across the 14 soil sample locations (N = 308).

3.5 Morphological diversity

Aerial mycelium colour was recorded for all 308 isolates. White (14.6%), mixed (12.7%), grey (12.7%), black (13.0%) and creamish (11.0%) morphotypes were the most prevalent, while blue (1.3%) and red (1.9%) morphotypes were the least common (Table 4, Figure 3). Percentages in Table 4 are calculated directly from the reported isolate counts against N = 308 and differ marginally (≤0.8 percentage points) from the percentages given in the source dataset — see Editorial Note on the title page.

Aerial mycelium colour	No. of isolates	% of total (recalculated)
White	45	14.61
Grey	39	12.66
Brown	36	11.69
Black	40	12.99
Pinkish	18	5.84
Red	6	1.95
Yellow	13	4.22
Green	28	9.09
Blue	4	1.30
Orange	6	1.95
Creamish	34	11.04
Mixed	39	12.66
Total	308	100.00

Table 4. Aerial mycelium pigmentation among the 308 actinomycete isolates.

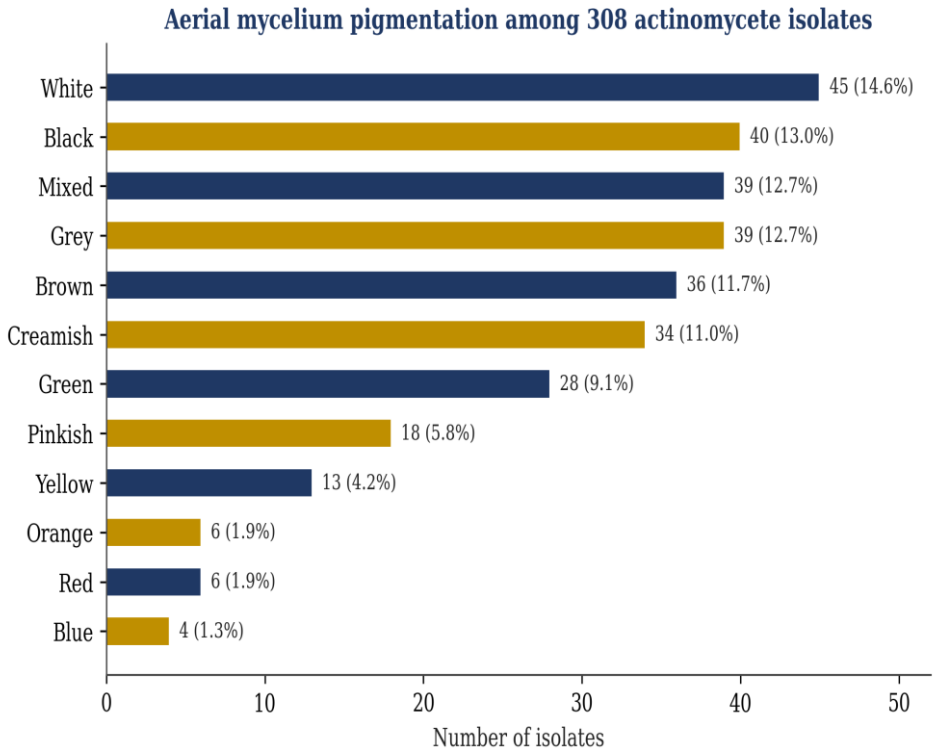

Figure 3. Aerial mycelium pigmentation distribution among the 308 actinomycete isolates.

3.6 Screening for compactin-to-pravastatin bioconversion

All 308 isolates were screened by TLC-based whole-cell bioconversion assay. The large majority of isolates (283/308, 91.9%) showed weak-to-moderate reaction intensity (+ or ++), 23 isolates (7.5%) showed strong intensity (+++), and only two isolates — G4 and G7, both recovered from Sangvi village soil (site G, Akkalkot–Solapur) — showed the highest reaction intensity (++++), indicating markedly superior compactin hydroxylation activity relative to the rest of the collection (Figure 4). These two isolates were carried forward for fermentation parameter optimization.

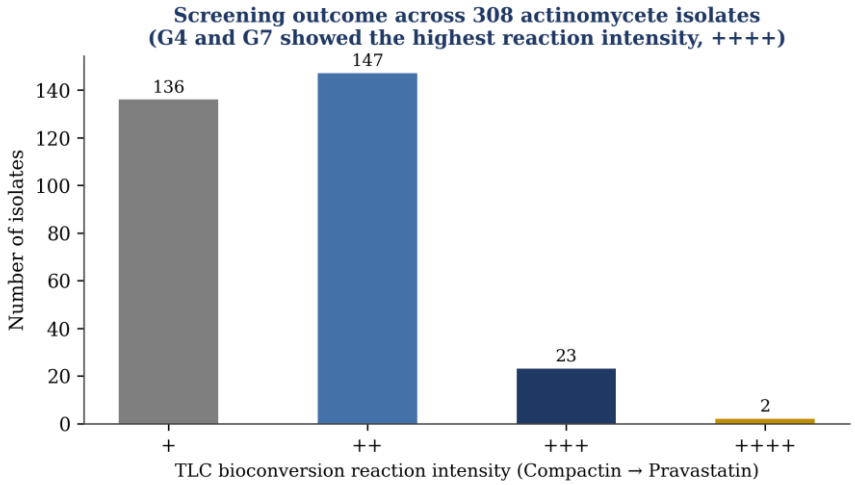

Figure 4. Distribution of TLC bioconversion reaction intensity across all 308 screened isolates.

3.7 Fermentation parameter optimization for isolates G4 and G7

Among the five media tested, starch–casein–dextrose broth (SCDM) gave the highest bioconversion activity for both G4 and G7, followed by CSP-YE complex medium; M9 basal medium performed poorest. Optimum bioconversion activity was observed at pH 7.0–7.5, with reduced activity at both more acidic (pH 6.0) and more alkaline (pH 8.0) conditions. Temperature optimization identified 28 °C as optimal, with reduced activity at 25 °C and at 30–32 °C (Figure 5). A visible dark-brown pigmentation developed in production flasks by 144 h, consistent with active secondary metabolite production preceding the 216 h harvest point.

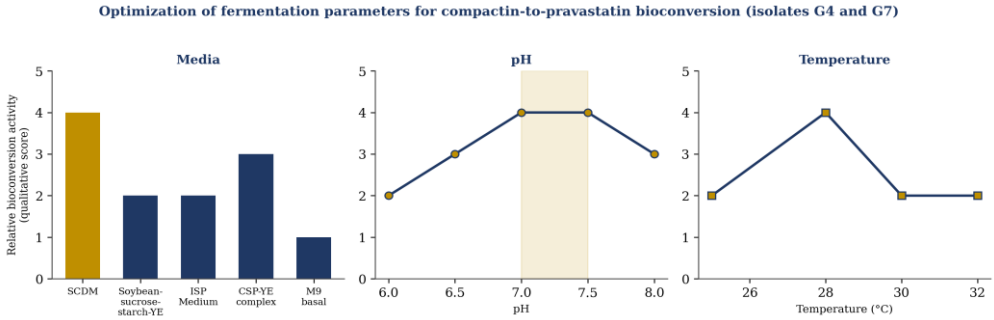

Figure 5. Optimization of (a) fermentation medium, (b) pH, and (c) temperature for compactin-to-pravastatin bioconversion by isolates G4 and G7.

4. DISCUSSION

The present study demonstrates that a systematic combination of selective medium optimization, antibiotic supplementation and physicochemical pretreatment substantially improves the recovery of culturable actinomycetes from diverse, under-explored agro-industrial soils. The superior performance of yeast extract–malt extract–dextrose agar over starch casein, glycerol asparagine, and oatmeal agar is consistent with earlier reports that mycelial-producing actinomycetes, particularly Streptomyces-type morphotypes, are efficiently recovered on nutrient-rich, moderately complex media [2,11].

The finding that moderate dry heat (40 °C) outperformed higher temperatures (50–70 °C) for isolate recovery mirrors observations that while heat pretreatment selects for spore-forming actinomycetes by suppressing vegetative bacterial competitors, excessive heat can also inactivate a proportion of the actinomycete spore population itself, particularly of less thermotolerant genera [2,6]. Similarly, the synergistic effect of combining dry heat with calcium carbonate and phenol pretreatment observed here parallels the broader literature on soil pretreatment for actinomycete enrichment, in which calcium salts are thought to support spore stability and germination while phenol selectively suppresses fast-growing bacterial and fungal competitors [2,6].

The concentration of isolate recovery in black and brown soils, and specifically the high yield from the Sangvi village site (34 isolates, and the source of both top-performing isolates G4 and G7), is consistent with reports that actinomycete abundance and bioactive potential tend to be higher in organic-matter-rich, less-disturbed agricultural soils compared with industrially contaminated or oil-bearing soils [12–14]. This reinforces the value of systematically sampling a range of under-explored habitat types when bioprospecting for high-value biocatalytic strains, rather than restricting sampling to conventional agricultural soils alone.

The identification of isolates G4 and G7 as high-intensity compactin-to-pravastatin bioconverters is notable given that only 2 of 308 isolates (0.6%) reached the highest reaction-intensity category, underlining both the rarity of high-efficiency biocatalytic strains within a natural actinomycete population and the value of large-scale primary

screening. The optimal fermentation conditions identified here — SCDM medium, pH 7.0–7.5, 28 °C — are broadly consistent with reported optimum conditions (pH 6.0–9.0, 25–32 °C) for microbial compactin bioconversion by Streptomyces and related genera [7,15], and support the feasibility of scaling this bioconversion for isolates G4 and G7. As no molecular identification data were available in the source dataset, definitive genus/species assignment of G4 and G7, together with confirmation of the specific hydroxylase system responsible for their activity (e.g., a cytochrome P450 system as in S. carbophilus, or a constitutive hydroxylase as in Actinomadura sp. [8,9]), remains an important next step.

5. CONCLUSION

Systematic optimization of isolation medium, antibiotic supplementation and soil pretreatment enabled recovery of 308 morphologically diverse actinomycete isolates from 14 soil habitats of the Solapur–Raichur region. Screening this collection for compactin-to-pravastatin bioconversion activity identified two isolates, G4 and G7, with markedly superior bioconversion capacity under optimized fermentation conditions (SCDM medium, pH 7.0–7.5, 28 °C). These isolates represent promising candidates for further molecular identification, enzyme-level characterization, and scale-up as industrial biocatalysts for pravastatin production.

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Author Contributions

Sidhant Kawade: sample collection, isolation, screening, data analysis, and manuscript drafting. Prof. Dr. L. H. Kamble: study conception, supervision, resources, and manuscript review and editing. [Author to confirm/adjust contribution statement as per journal CRediT taxonomy requirements.]

Conflict of Interest

The authors declare no conflict of interest.

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