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EXPLORATION OF ACTINOMYCETES DIVERSITY IN DIVERSE SOILS OF SOLAPUR–RAICHUR REGION AND OPTIMIZATION OF CULTURE CONDITIONS FOR MICROBIAL TRANSFORMATION OF COMPACTIN INTO PRAVASTATIN

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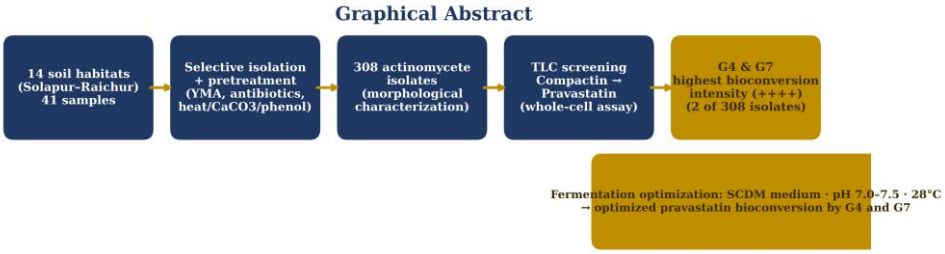
Graphical Abstract



Workflow summary: soil sampling → selective isolation and pretreatment → 308 actinomycete isolates → TLC bioconversion screening → identification of isolates G4 and G7 → fermentation optimization for pravastatin bioconversion.

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

1.1 Actinomycetes as a Source of Bioactive Metabolites

Actinomycetes are Gram-positive, filamentous, spore-forming bacteria belonging to the phylum Actinobacteria, characterized by a branching mycelial growth habit reminiscent of filamentous fungi and a high genomic GC content. They are ubiquitously distributed across terrestrial and extreme habitats — from agricultural soils and

compost to deserts, caves, hypersaline environments and marine sediments — and are recognized as one of the richest natural sources of bioactive secondary metabolites of pharmaceutical and industrial significance [1,12]. Of the roughly 22,250 bioactive microbial natural products documented to date, actinomycetes are estimated to contribute close to half, and approximately three-quarters of clinically used antibacterial agents trace their origin to this group [1]. Beyond antibiotics, actinomycetes are prolific producers of antifungal, antitumor, immunosuppressive and enzyme-inhibitory compounds, as well as industrial enzymes, biopesticides and enzyme inhibitors used in cholesterol-lowering therapeutics [1,12].

Soil remains the principal reservoir of culturable actinomycetes, and their distribution and diversity are strongly influenced by soil type, organic matter content, pH, moisture, and the extent and nature of anthropogenic activity in the surrounding habitat. Streptomyces is by far the most frequently isolated genus from soil, but a wide range of 'rare' actinomycete genera — including Actinomadura, Nocardia, Micromonospora, Nocardiopsis and Streptosporangium — are also recovered under appropriately selective conditions, and these rare genera are increasingly recognized as a comparatively under-exploited reservoir of structurally novel bioactive chemistry, since the frequency of discovering genuinely novel compounds from readily culturable Streptomyces has declined in recent decades as this genus has been progressively over-mined [11].

1.2 Selective Isolation Strategies

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, Nonomura and co-workers over several decades established that specialized isolation media (such as humic acid–vitamin agar), targeted antibiotic supplementation, and physical or chemical pretreatment of soil samples prior to plating markedly improve the selective recovery of actinomycetes, including rare and slow-growing genera, from soil [2–5]. Physical pretreatments such as air-drying and controlled dry-heating exploit the comparatively heat- and desiccation-resistant spores of many actinomycete genera to selectively deplete vegetative bacterial and fungal competitors, while chemical pretreatments such as phenol and calcium carbonate act through complementary mechanisms — phenol as a general biocide that spares resistant actinomycete spores, and calcium salts as a support for spore stability, sporulation and subsequent germination [2,6]. These principles, first developed for Japanese agricultural soils, have since been validated and extended across a wide range of habitat types, including forest soils of northeast India [11], the Western Ghats biodiversity hotspot [12], Kashmir Himalayan soils [13], and even karstic cave environments [6], underscoring their general applicability across diverse and geographically distinct soil ecosystems.

Because no single medium, antibiotic combination or pretreatment strategy is universally optimal for every soil type or target genus, empirical optimization for the specific soils and geographic region under study — as undertaken in this work — remains an essential first step in any actinomycete bioprospecting programme, particularly when the downstream objective is the discovery of strains with a specific, high-value biocatalytic activity rather than simple diversity documentation [14].

1.3 Pravastatin and the Pharmaceutical Importance of Compactin Bioconversion

Statins are among the most widely prescribed drug classes worldwide, acting as competitive inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme in hepatic cholesterol biosynthesis, and are a cornerstone of the clinical management of hypercholesterolemia and cardiovascular disease risk. Pravastatin, one of the earliest statins brought to market, is distinguished from most other statins in that it is produced industrially not by total chemical synthesis but by a two-step fermentation-and-biotransformation process: compactin (also known as mevastatin or ML-236B) is first produced by fermentation of the fungus Penicillium citrinum, and the resulting compactin is then regio- and stereoselectively 6β-hydroxylated to pravastatin by whole-cell microbial biocatalysis, most commonly using Streptomyces carbophilus at industrial scale [7,9]. This microbial hydroxylation route is strongly preferred over chemical synthesis, which is costly and generates unwanted stereoisomeric by-products that complicate downstream purification [7].

A number of actinomycete genera and species beyond S. carbophilus have been reported to carry out this same bioconversion with varying efficiency and via distinct enzymatic mechanisms: Actinomadura sp. strain 2966 and Actinomadura sp. ATCC 55678 possess a constitutive, NADPH-dependent hydroxylase distinct from the inducible cytochrome P450 system of S. carbophilus [8,10]; Streptomyces sp. Y-110, isolated directly from soil, has been shown to achieve substantial bioconversion yields in batch culture [7]; and more recently, cytochrome P450 systems such as CYP105D7 from Streptomyces avermitilis have been characterized for the same hydroxylation reaction [9]. This diversity of hydroxylase systems across unrelated actinomycete lineages suggests that the capacity for compactin bioconversion, while relatively rare within a given natural population, may be recoverable from a range of previously unscreened soil isolates — motivating the systematic, large-scale primary screening approach adopted in the present study, rather than restricting the search to previously reported producer species alone.

1.4 Study Rationale and Objectives

Despite the well-established industrial and clinical importance of microbial compactin bioconversion, the actinomycete diversity of the Solapur–Raichur belt of Maharashtra and Karnataka — an agriculturally and industrially heterogeneous region encompassing sugarcane, oil, milk and fertilizer processing industries alongside conventional agricultural and riverine soils — has not been systematically characterized for this specific biocatalytic activity. The present study was therefore undertaken with four specific objectives: (i) to isolate and

morphologically characterize culturable actinomycetes from 14 diverse soil habitats of this region; (ii) to empirically optimize the isolation medium, antibiotic supplementation and physical/chemical pretreatment strategy for maximal, contamination-free recovery from these specific soils; (iii) to screen the resulting isolate collection for compactin-to-pravastatin bioconversion activity by a whole-cell thin-layer chromatography assay; and (iv) to optimize fermentation medium, pH and temperature for the isolate(s) identified as most active, as a first step toward their potential development as industrial biocatalysts.

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.

Table 1. Soil Sample Collection Sites and Habitat Description

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

2.2 Selective Isolation Medium

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.

2.3 Antibiotic Concentration Optimization

Following medium selection, antibiotic concentrations were optimized on YMA using black soil sample A. An initial screen compared the control antibiotic combination against single-antibiotic increases (added cycloheximide, fluconazole, or rifampicin at 50 µg/mL, and a combined rifampicin 25 µg/mL + fluconazole 30 µg/mL trial). A second, refined round of optimization then compared four combinations at higher overall concentrations to identify the combination giving maximal isolate recovery with zero visible contamination.

2.4 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), each evaluated independently. 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].

2.5 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.

2.6 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.

2.7 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.

2.8 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) (Table 2, Figure 1). YMA was therefore selected as the isolation medium for the full study.

Table 2. Comparison of Isolation Media for Actinomycete Recovery (Black Soil Sample, Five Replicate Plates)

Medium	Isolates recovered
Yeast extract–malt extract–dextrose agar (YMA)	13 (selected)
Starch casein agar	10
Glycerol asparagine agar	10
Oatmeal agar	7

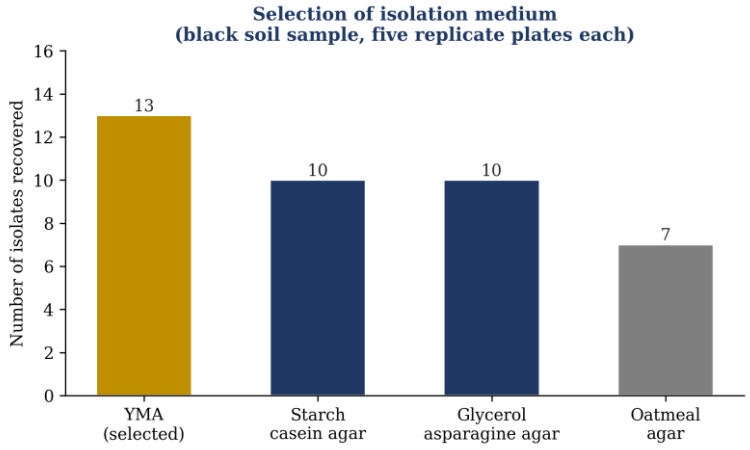


Figure 1. Comparison of isolation media for actinomycete recovery.

3.2 Antibiotic Concentration Optimization

In the absence of antibiotics, heavy bacterial and fungal contamination was observed across all plates. The initial optimization round (Table 3) showed that raising individual antibiotic concentrations (cycloheximide, fluconazole or rifampicin to 50 µg/mL) reduced but did not eliminate contamination, with the fluconazole 50 µg/mL trial giving the highest isolate count (13) but residual bacterial contamination. A second, refined round (Table 4) 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; this combination was selected for the full study (Figure 2).

Table 3. Preliminary Antibiotic Concentration Screening (Round 1)

Antibiotic combination (µg/mL)	Isolates	Observation
No antibiotics	2	Contamination in each plate
Cyclo 25, Flu 20, Nys 25, Rif 25 (control)	10	Few plates contaminated
Control + cycloheximide 50	8	Few plates contaminated
Control + fluconazole 50	13	Few plates with bacterial contamination
Control + rifampicin 50	11	Few plates contaminated
Control + rifampicin 25 + fluconazole 30	13	Less contamination than other trials

Table 4. Refined Antibiotic Concentration Optimization (Round 2) — Final Combination Selected

Antibiotic combination (µg/mL)	Isolates	Observation
No antibiotics	—	Contamination in each plate
Cyclo 50, Flu 50, Nys 50, Rif 50	6	No contamination; slow isolate growth
Cyclo 25, Flu 50, Nys 25, Rif 25	10	No contamination
Cyclo 25, Flu 50, Nys 25, Rif 50 (selected)	13	No contamination

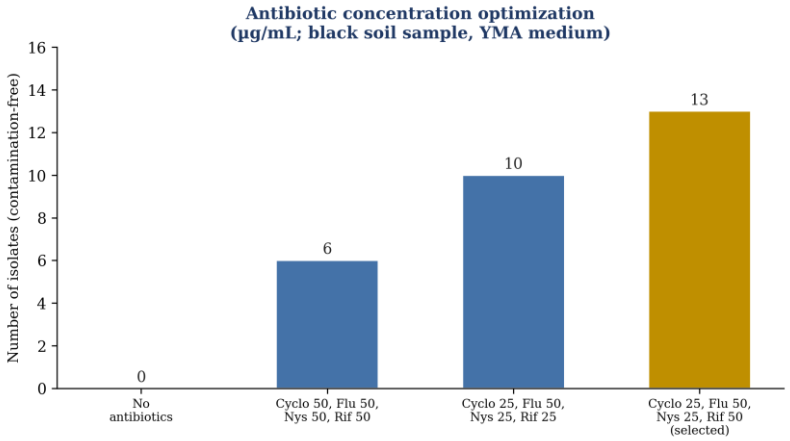


Figure 2. Refined antibiotic concentration optimization — isolate recovery under increasing antibiotic stringency.

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 (Table 5). Centrifugation at 4000 rpm for 10 min modestly improved recovery (10 isolates) over the untreated control (9 isolates). Phenol at 0.5–1.0% (14 isolates each) and calcium carbonate at 0.5% (14 isolates) each independently enhanced isolate recovery over untreated soil (11 and 10 isolates respectively) (Tables 6–7, Figure 3). 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) among all conditions tested (Table 8, Figure 4), confirming that physical and chemical enrichment act complementarily rather than redundantly.

Table 5. Effect of Dry Heat and Centrifugation Pretreatment (Soil Sample A)

Treatment	Isolates
Dry heat, 40 °C, 30 min	14
Dry heat, 50 °C, 30 min	9
Dry heat, 60 °C, 30 min	8
Dry heat, 70 °C, 30 min	0
Control (no treatment)	9
Centrifugation, 4000 rpm, 5 min	9

Treatment	Isolates
Centrifugation, 4000 rpm, 10 min	10

Table 6. Effect of Phenol Pretreatment (Soil Sample A)

Phenol concentration	Isolates
Control (no treatment)	11
0.5%	14
1.0%	14
1.5%	10
2.0%	10

Table 7. Effect of Calcium Carbonate Pretreatment (Soil Sample A)

Calcium carbonate concentration	Isolates
Control (no treatment)	10
0.5%	14
1.0%	13
1.5%	10
2.0%	12

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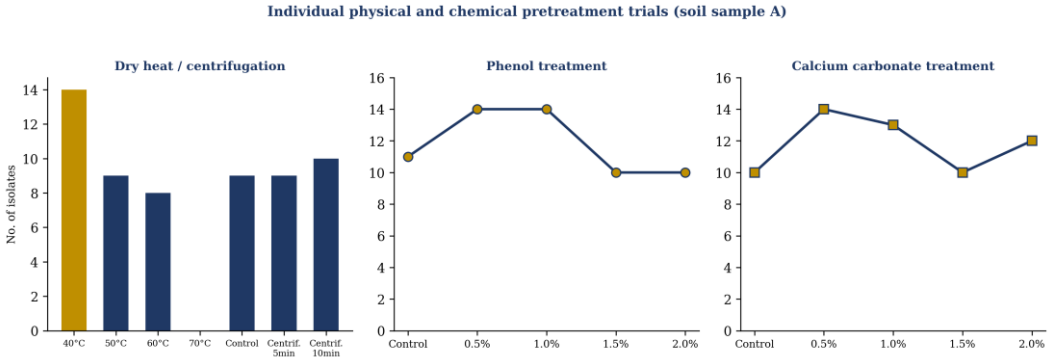

Figure 3. Individual dose–response trials for physical (heat/centrifugation) and chemical (phenol, calcium carbonate) pretreatments.

Table 8. Combined Physical and Chemical Pretreatment Trial (Soil Sample A)

Treatment combination	Isolates
Control (no treatment) (C)	8
Dry heat 40 °C + Phenol 0.5% (C1)	9
Dry heat 40 °C + $CaCO_3$ 0.5% (C2)	12
Dry heat 40 °C + $CaCO_3$ 0.5% + Phenol 0.5% (C3) — selected	16

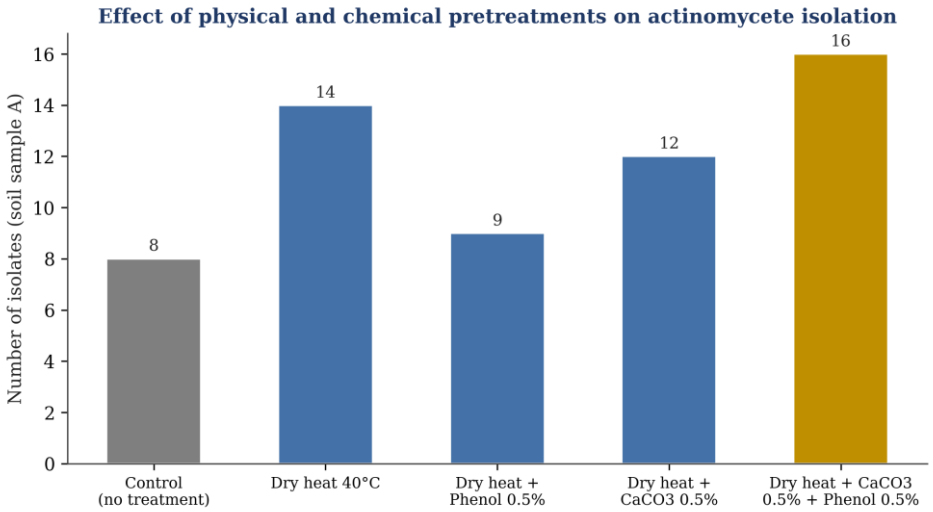

Figure 4. Comparison of combined pretreatment strategies — the dry heat + $CaCO_3$ + phenol combination gave maximal isolate recovery.

Distinct pigmentation patterns were observed among colonies recovered under calcium carbonate versus phenol treatment when applied separately, with limited overlap between the morphotypes favoured by each treatment — indicating that each pretreatment selectively enriches for a partially distinct sub-population of the soil actinomycete community rather than simply improving recovery of the same dominant morphotypes.

3.4 Diversity and Distribution of Isolates Across Soil Samples

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 9, Figure 5).

Table 9. Distribution of the 308 Actinomycete Isolates Across the 14 Soil Sample Sites

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

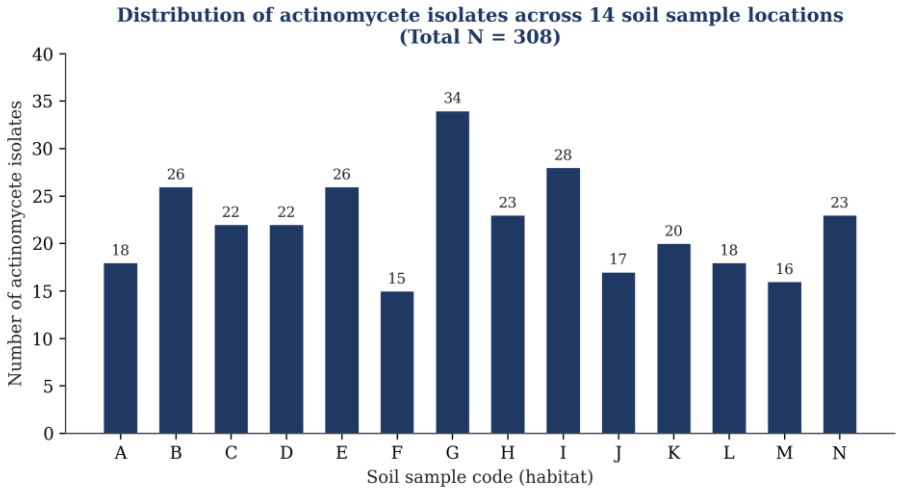

Figure 5. Distribution of actinomycete isolates across the 14 soil sample locations (N = 308).

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 10, Figure 6). Percentages in Table 10 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. All isolates were confirmed as actinomycetes by dry, powdery colony morphology, Gram-positive staining, and microscopic observation of branching filamentous mycelium bearing spores and hyphae — features distinctive of the group and consistent with the standard criteria used to differentiate actinomycetes from unicellular bacterial contaminants.

Table 10. Aerial Mycelium Pigmentation Among the 308 Actinomycete Isolates

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

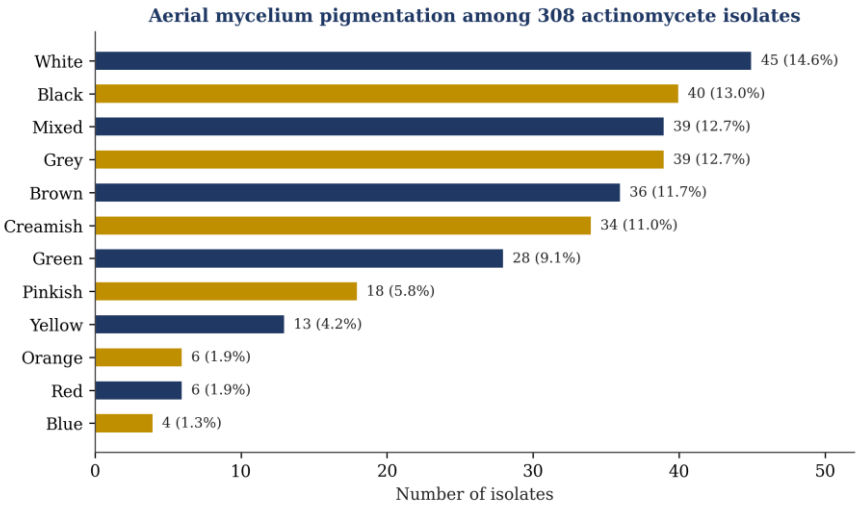

Figure 6. Aerial mycelium pigmentation distribution among the 308 actinomycete isolates.

3.6 Submerged Fermentation and Metabolite Production

All 308 isolates were grown in submerged fermentation, with seed cultures transferred into production medium and incubated for 216 h at 28 °C, 220 rpm. Production flasks showed visible dark-brown pigmentation by 144 h relative to the near-colourless appearance at 48 h, a change consistent with active secondary metabolite production preceding the fixed 216 h harvest point used for downstream screening.

3.7 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 7). These two isolates, representing just 0.6% of the total screened collection, were carried forward for fermentation parameter optimization.

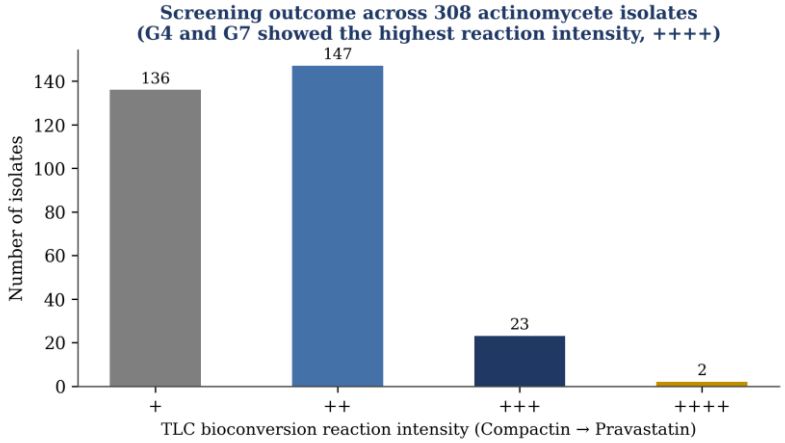

Figure 7. Distribution of TLC bioconversion reaction intensity across all 308 screened isolates.

3.8 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; the soybean–sucrose–starch–yeast extract and ISP media gave intermediate activity, and M9 basal medium performed poorest (Table 11). 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 (Table 12). Temperature optimization identified 28 °C as optimal, with reduced activity at 25 °C and at 30–32 °C (Table 13, Figure 8).

Table 11. Fermentation Medium Optimization for Isolates G4 and G7 (Qualitative TLC-Based Ranking)

Medium	Relative bioconversion activity
Starch–Casein–Dextrose broth (SCDM) — selected	Highest
CSP-YE complex medium	High

Medium	Relative bioconversion activity
Soybean–sucrose–starch–YE medium	Moderate
ISP medium	Moderate
M9 basal medium	Lowest

Table 12. pH Optimization for Isolates G4 and G7

pH	Relative bioconversion activity
6.0	Low
6.5	Moderate
7.0	Highest
7.5	Highest
8.0	Moderate

Table 13. Temperature Optimization for Isolates G4 and G7

Temperature (°C)	Relative bioconversion activity
25	Low
28	Highest
30	Low
32	Low

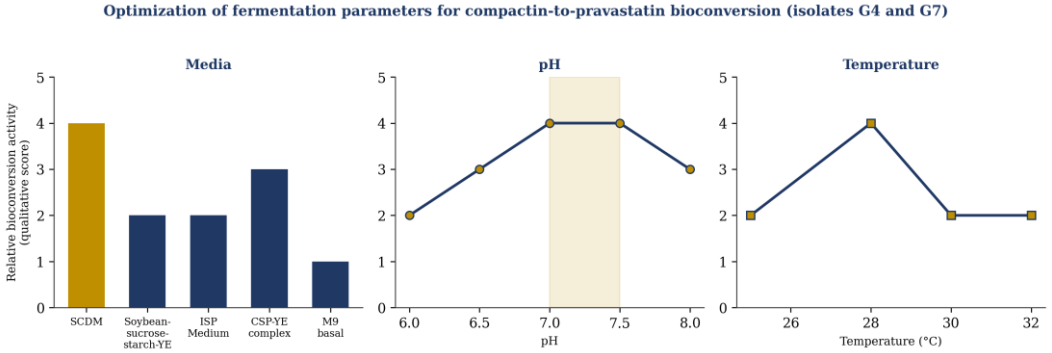

Figure 8. Optimization of (a) fermentation medium, (b) pH, and (c) temperature for compactin-to-pravastatin bioconversion by isolates G4 and G7.

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 distinct pigmentation patterns observed under calcium carbonate versus phenol pretreatment applied separately further support the interpretation that these two chemical pretreatments enrich for partially non-overlapping sub-populations of the soil actinomycete community, reinforcing the rationale for combining rather than substituting between pretreatment strategies.

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.

Conflict of Interest

The authors declare no conflict of interest.

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