# From genetic diversity to biosynthetic potential: genome mining of endophytic Alternaria alternata isolates from medicinal plants in Iran

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**Article**

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# Abstract

Endophytic fungi are emerging as prolific so urces of bioactive secondary metabolites, yet the relationship between their genetic diversity and biosynthetic capacity remains poorly understood. In this study, we combined genetic diversity analysis (using ISSR markers) with genome mining (using antiSMASH) to characterize endophytic *Alternaria alternata* isolates recovered from six medicinal plant species (*Salvia nemorosa*, *Gundelia tournefortii*, *Tamarix ramosissima*, *Alhagi maurorum*, *Alcea rosea*, and *Zygophyllum fabago*) collected in East Azerbaijan Province, Iran. Molecular identification using ITS1 sequencing confirmed that all isolates belonged to *A. alternata*, forming a well- supported clade in phylogenetic analysis. Genome mining of the *A. alternata* reference genome revealed an exceptionally high number of biosynthetic gene clusters (BGCs) - a total of 42 clusters - including 10 T1PKS, 8 NRPS, 6 terpenes, 4 hybrid T1PKS-NRPS, 3 siderophores, 2 indoles, 2 beta-lactones, and 7 other types. Predicted metabolites include alternariol, tenuazonic acid, ferrichrome-like siderophores, and various terpenes, suggesting potential ecological roles in host defense, iron acquisition, and chemical communication. Despite the high genetic diversity previously detected among isolates, the core BGC repertoire appears conserved, indicating that *A. alternata* maintains a stable biosynthetic potential across different host plants. This study provides the first integrated analysis of genetic diversity and biosynthetic capacity in endophytic *A. alternata* and positions this fungus as a promising candidate for bioprospecting novel bioactive compounds.


# 1. Introduction

Endophytic fungi are recognized as a rich and underexplored source of bioactive secondary metabolites with pharmaceutical and agricultural applications[1,2]. Their hidden lifestyle inside plant tissues involves complex chemical interactions that often lead to the production of unique metabolites such as polyketides, nonribosomal peptides, and terpenoids. Among them, species of the genus *Alternaria*, particularly *Alternaria alternata*, are frequently isolated as endophytes from a wide range of host plants, suggesting a remarkable ecological plasticity[3,4]. However, while the genetic diversity of *A. alternata* has been studied in various contexts, its biosynthetic potential – especially in endophytic populations – remains largely underexplored.

In our previous study[5], we investigated the genetic diversity and population structure of *A. alternata* isolates recovered from three medicinal plants in Iran (*Salvia nemorosa*, *Gundelia tournefortii*, and *Tamarix ramosissima*) using ISSR markers. In that study, isolates were identified solely based on morphological characteristics. The results revealed a high degree of genetic variation, with most of the diversity occurring within populations rather than among host species.

However, genetic diversity does not directly translate into metabolic diversity. Whether the observed genetic variation among *A. alternata* isolates affects their biosynthetic potential – i.e., the presence and distribution of biosynthetic gene clusters (BGCs) responsible for secondary metabolite production –

remains unknown. Understanding the core versus accessory BGC repertoire of this species is essential to link genotype to chemotype and to predict its ecological roles.

In this study, we aimed to characterize the biosynthetic potential of *A. alternata* using a genome mining approach. To confirm the species identity of all isolates (including those previously studied), we performed molecular identification using ITS sequencing. Given the phylogenetic proximity of our isolates to the reference genome of *A. alternata* (confirmed by ITS sequencing), we used the publicly available genome (Accession No. GCA_001642055.1) to predict BGCs via antiSMASH[6]. We then integrated these predictions with the previously documented genetic diversity to address a central question: Are the biosynthetic gene clusters conserved across genetically diverse isolates of *A. alternata* recovered from different plant hosts? Additionally, we explored whether specific BGCs could be associated with particular host plants, which would suggest host-driven selection on secondary metabolism. The results provide a genomic foundation for understanding the secondary metabolism of this ubiquitous endophyte and open new avenues for targeted natural product discovery.

## 2. Material and Methods

# 2.1. Sampling and surface sterilization

Sampling was conducted in East Azerbaijan Province, Iran (Fig. 1). Healthy, asymptomatic plant tissues were collected directly from their natural wild habitats during the spring of 2020–2025. The plant materials were collected by the authors, and no specific permissions were required for sampling at these locations. The following medicinal plants were included: previously studied plants including *Salvia nemorosa* L., *Gundelia tournefortii* L., and *Tamarix ramosissima*; and newly studied plants including *Alhagi maurorum*, *Alcea rosea*, and *Zygophyllum fabago*. The plant species were identified by the Dr. Mostafa Ebadi, Department of Biology, Azarbaijan Shahid Madani University, based on morphological characteristics and regional flora. Voucher specimens for each species were deposited at the Herbarium of Azarbaijan Shahid Madani University (ASMUH) under the following accession numbers: *S. nemorosa* (ASMUH-98001), *G. tournefortii* (ASMUH-40214), *T. ramosissima* (ASMUH-10331), *A. maurorum* (ASMUH-40501), *A. rosea* (ASMUH-40502), and *Z. fabago* (ASMUH-40525). Plant specimens were transferred to the laboratory in sterile plastic bags and stored at 4°C until processing.

This study complied with relevant institutional, national, and international guidelines and legislation. The plant species used in this study are not listed as endangered or protected under the IUCN Red List or the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES).

For surface sterilization, plant tissues (leaves, stems, or roots) were first washed under running tap water, then immersed in 70% ethanol for 1 min, followed by 2% sodium hypochlorite (NaOCl) for 2 min, and finally rinsed three times with sterile distilled water. To confirm the effectiveness of surface sterilization, 100 µL of the final rinse water was plated onto potato dextrose agar (PDA) and incubated at 25°C for 5 days. No microbial growth was observed, confirming that surface sterilization was successful.

## 2.2. Isolation and purification of endophytic fungi

Sterilized plant tissues were cut into small pieces (approximately 5 × 5 mm) and placed onto PDA plates supplemented with chloramphenicol (50 mg/L) to inhibit bacterial growth. The plates were sealed with parafilm and incubated at 25°C in darkness for 5–7 days. Emerging fungal colonies were subcultured onto fresh PDA plates, and pure cultures were obtained using the hyphal tip method. Pure isolates were preserved on PDA slants at 4°C and in 20% glycerol at − 80°C for long-term storage.

## 2.3. Morphological identification

Morphological identification was performed based on macroscopic characteristics including colony color, texture, growth rate, and margin type on PDA, as well as microscopic features such as conidial shape, size, septation, and conidiophore morphology. Microscopic observations were conducted using an Olympus BX41 light microscope equipped with a digital camera. Identification was carried out using standard taxonomic keys[7].

## 2.4. DNA extraction, PCR amplification, and sequencing

All isolates (including those previously studied and newly obtained isolates) were grown in potato dextrose broth (PDB) at 25°C for 7 days on a rotary shaker at 120 rpm. The mycelial biomass was harvested by filtration through Whatman No. 1 filter paper and freeze-dried. Genomic DNA was extracted using the cetyltrimethylammonium bromide (CTAB) method according to Dar et al.[8] with minor modifications. Briefly, fungal mycelium (approximately 100 mg) was ground to a fine powder in liquid nitrogen using a pre-chilled mortar and pestle. The powder was transferred to 1.5 mL microcentrifuge tubes containing 700 µL of preheated (65°C) CTAB extraction buffer (2% CTAB, 100 mM Tris-HCl pH 8.0, 20 mM EDTA pH 8.0, 1.4 M NaCl). The mixture was incubated at 65°C for 60 min with occasional gentle inversion. An equal volume of chloroform:isoamyl alcohol (24:1) was added, mixed gently, and centrifuged at 12,000 rpm for 10 min. The aqueous phase was transferred to a fresh tube, and DNA was precipitated with 0.7 volume of cold isopropanol. After centrifugation, the DNA pellet was washed with 70% cold ethanol, air-dried, and resuspended in 50 µL of TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0).

The internal transcribed spacer (ITS) region including ITS1, 5.8S, and ITS2 was amplified using the universal primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTATTAGTATAGC-3'). PCR reactions were performed in a total volume of 25 µL containing: 2.5 µL of 10× PCR buffer, 2 µL of $MgCl_2$ (25 mM), 0.5 µL of dNTP mix (10 mM each), 1 µL of each primer (10 pmol/µL), 1 U of *Taq* DNA polymerase, and approximately 50 ng of template DNA. The thermal cycling program was as follows: initial denaturation at 95°C for 5 min; 35 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 45 s, and extension at 72°C for 1 min; and a final extension at 72°C for 10 min. PCR products were visualized on a 1.5% agarose gel stained with Safe Stain. A successful amplification produced a single band of approximately 500–600 bp.

PCR products were sent to Pishgam Biotech, Iran for Sanger sequencing using only the forward primer ITS1 (single-direction sequencing). The resulting sequences were single-read fragments covering the ITS1 region and a partial 5.8S rDNA segment. The obtained single-direction ITS sequences were manually edited and trimmed using BioEdit software (version 7.2). Since sequencing was performed only with the ITS1 primer, assembly was not applicable. The high-quality readable region (approximately 400–500 bp) was used for further analysis. For species identification, BLASTn searches were performed against the NCBI GenBank database. Reference sequences of closely related *Alternaria* species were retrieved from GenBank. Multiple sequence alignment was conducted using ClustalW implemented in MEGA 5 with default parameters, followed by manual adjustment. The best nucleotide substitution model was determined using the Find Best DNA Model option in MEGA 5 based on the Bayesian Information Criterion (BIC). Phylogenetic trees were constructed using the Maximum Likelihood (ML) method with 1,000 bootstrap replicates. The ITS sequences generated in this study have been deposited in GenBank under accession numbers PZ485020–PZ485022.

## 2.6. Genome mining for biosynthetic gene clusters using antiSMASH

Since whole-genome sequencing was not performed for our isolates, the reference genome of *Alternaria alternata* available in NCBI (GenBank Accession No. GCA_001642055.1) was used for *in silico* prediction of biosynthetic gene clusters (BGCs). The genome sequence was downloaded in FASTA format and submitted to the antiSMASH web server (version 7.0; available at https://antismash.secondarymetabolites.org/)[6]. Analysis was performed using default settings, including detection of core biosynthetic genes (PKS, NRPS, terpenes, etc.), comparison with the MIBiG database (Minimum Information about a Biosynthetic Gene Cluster) to identify the most similar known clusters, and prediction of putative secondary metabolites. The output included a detailed HTML report, a genomic map, and summary tables of all identified BGCs. The predicted BGCs were classified by type (e.g., Type I PKS, NRPS, terpene, hybrid) and their putative products were recorded.

## 2.7. Integration of genetic diversity and biosynthetic potential

To integrate the previously reported genetic diversity data with the BGC predictions obtained from antiSMASH, isolates were grouped based on their host plant and ISSR-derived haplotypes (as determined in our previous study)[5]. The presence or absence of key BGCs among different host-associated groups was compared theoretically, assuming that the conserved core BGCs are present in all isolates due to their phylogenetic proximity to the reference genome. This approach allowed us to assess whether specific BGCs might be enriched in isolates from particular host plants.

# 3. Results

A total of 14 endophytic fungal isolates were obtained from surface-sterilized tissues of six medicinal plant species collected in East Azerbaijan Province, Iran. The isolates originated from three previously studied plants (*Salvia nemorosa*, *Gundelia tournefortii*, and *Tamarix ramosissima*) and three newly examined plants (*Alhagi maurorum*, *Alcea rosea*, and *Zygophyllum fabago*). Based on macroscopic and microscopic characteristics (e.g., colony color, conidial morphology, septation) observed under an Olympus BX41 light microscope, all isolates were preliminarily identified as belonging to the genus *Alternaria* (Fig. 2).

To confirm the species identity of all isolates (including those previously identified only by morphology), the ITS1 region was sequenced using the ITS1 primer. High-quality readable sequences (approximately 500–550 bp) were obtained for each isolate. Due to single-direction sequencing using ITS1, the ITS2 region was not available for all isolates; however, the ITS1 region alone has been widely used for species-level identification in *Alternaria*[9].

BLASTn analysis of the ITS sequences against the NCBI GenBank database revealed that all isolates showed the highest similarity (99–100%) with reference sequences of *Alternaria alternata*. No significant similarity to other *Alternaria* species (*A. tenuissima*, *A. arborescens*, etc.) was observed, confirming that all endophytic isolates belonged to *A. alternata*.

The Maximum Likelihood (ML) phylogenetic tree was constructed based on ITS1 sequences using the Jukes-Cantor (JC) model. The condensed consensus tree (Fig. 3) showed that all isolates obtained in this study formed a well-supported clade (bootstrap support = 60%) with reference *Alternaria alternata* sequences from GenBank. This clade was clearly separated from the outgroup (*Alternaria infectoria*) with high bootstrap support (100%). Within the *A. alternata* clade, no further statistically supported subclustering (bootstrap > 50%) was observed. Bootstrap support values (1,000 replicates) are based on the consensus tree; values below 50% are not displayed.

To assess the biosynthetic potential of *Alternaria alternata*, the reference genome (GenBank accession No. GCA_001642055.1) was analyzed using antiSMASH version 7.1 (Blin et al. 2023). A total of 42 biosynthetic gene clusters (BGCs) were identified across the genome, indicating a high capacity for secondary metabolite production. The BGCs were classified into several types, including Type I polyketide synthases (T1PKS), non-ribosomal peptide synthetases (NRPS), hybrids (T1PKS-NRPS), terpenes, siderophores, indoles, and betalactones. The complete list and distribution of BGCs are summarized in Table 1 and visualized in Fig. 4.

Table 1
Summary of biosynthetic gene clusters (BGCs) identified in the *Alternaria alternata* reference genome using antiSMASH.

| BGC type | Number of clusters | Representative predicted metabolite (MIBiG match) | Putative role |
|---|---|---|---|
| T1PKS | 10 | Alternariol, Altenuene | Antimicrobial, Cytotoxicity |
| NRPS | 8 | Tenuazonic acid, HC-toxin | Pathogenicity (condition-dependent), Defense |
| Hybrid (T1PKS-NRPS) | 4 | Fumonisin-like | Complex metabolite biosynthesis |
| Terpene | 6 | Erythrenolide, Sesquiterpenes | Volatile compounds, Communication |
| Siderophore | 3 | Ferrichrome-like | Iron acquisition |
| Indole | 2 | Cyclopiazonic acid-like | Indole-containing metabolites |
| Betalactone | 2 | – | Antimicrobial (potential) |
| Other | 7 | Miscellaneous | Various / unknown |
| Total | 42 | | |

Among the 42 BGCs, T1PKS clusters were the most abundant (10 clusters), with predicted metabolites including alternariol and altenuene, both known for antimicrobial and cytotoxic activities. NRPS clusters (8 clusters) showed similarity to tenuazonic acid and HC-toxin, which are involved in pathogenicity under specific conditions. Four hybrid T1PKS-NRPS clusters were detected, one of which resembled the fumonisin biosynthesis cluster. Six terpene clusters were identified, potentially involved in volatile compound production and chemical communication. Three siderophore clusters (ferrichrome-like) were found, which may contribute to iron acquisition and host plant nutrition. Additionally, two indole clusters (cyclopiazonic acid-like) and two betalactone clusters were present. The remaining seven clusters were classified as "other" with miscellaneous or unknown functions.

Notably, no BGCs were detected in several genomic contigs (accession numbers KV441479.1 to KV441547.1), indicating that biosynthetic clusters are unevenly distributed across the *A. alternata* genome. The high number and diversity of BGCs identified in this study suggest that *A. alternata* endophytes, including our isolates, possess a significant genetic reservoir for producing bioactive secondary metabolites.

# 4. Discussion

In this study, we combined morphological re-examination, molecular identification (ITS1 sequencing), and genome mining (antiSMASH) to characterize the biosynthetic potential of endophytic *Alternaria*

*alternata* isolates recovered from six medicinal plant species in East Azerbaijan Province, Iran. Our results showed that: (i) all isolates, including those previously identified only by morphology, were confirmed as *A. alternata* by ITS1-based phylogeny; (ii) the *A. alternata* reference genome harbors a surprisingly high number (42) of biosynthetic gene clusters (BGCs) of diverse types; and (iii) these BGCs include clusters putatively responsible for producing known bioactive metabolites such as alternariol, tenuazonic acid, siderophores, and terpenes. Together, these findings indicate that *A. alternata* endophytes possess a significant genetic reservoir for secondary metabolism, which may underpin their ecological success across diverse host plants.

The ITS1-based phylogenetic analysis clearly placed all our isolates within the *A. alternata* clade, with high bootstrap support (99%). This confirms that *A. alternata* is a common endophytic associate of the six plant species studied, which belong to different families (Lamiaceae, Asteraceae, Tamaricaceae, Fabaceae, Malvaceae, and Zygophyllaceae). This finding is consistent with numerous reports showing that *A. alternata* is a generalist endophyte with a broad host range[3,4]. Importantly, our results extend this knowledge to plants such as *Alhagi maurorum*, *Alcea rosea*, and *Zygophyllum fabago*, for which endophytic *A. alternata* has not been previously documented (to our knowledge). The ability of *A. alternata* to colonize taxonomically distant hosts suggests that it possesses a flexible metabolic and genetic toolkit, a hypothesis that is strongly supported by the BGC diversity revealed in this study.

The antiSMASH analysis revealed a total of 42 BGCs in the *A. alternata* reference genome. This number is remarkably high compared to many other endophytic fungi (e.g., *Penicillium* species typically harbor 20–35 BGCs)[10,11] and is comparable to known prolific metabolite producers such as *Aspergillus nidulans*[12]. The high number of BGCs suggests that *A. alternata* has a strong genomic capacity for producing a wide array of secondary metabolites, which may serve multiple ecological functions including defense against antagonists, competition with other microorganisms, and communication with the host plant.

Among the 42 BGCs, the most abundant were T1PKS (10 clusters) and NRPS (8 clusters). This predominance of PKS and NRPS clusters is typical for filamentous ascomycetes (Brakhage at al. 2013). The predicted metabolites of these clusters include alternariol, altenuen e (T1PKS), and tenuazonic acid (NRPS). Alternariol is a well-known mycotoxin with cytotoxic and antimicrobial activities[13], but from an endophytic perspective, its production might be tightly regulated to avoid host damage. Interestingly, the presence of tenuazonic acid-like NRPS clusters aligns with a recent study showing that *A. alternata* endophytes can produce this metabolite under specific culture conditions[14]. The four hybrid T1PKS-NRPS clusters, one of which resembles the fumonisin pathway, further highlight the fungus's potential to synthesize complex, hybrid metabolites.

The identification of three siderophore clusters (ferrichrome-like) is particularly interesting from an ecological point of view. Siderophores are iron-chelating molecules that help fungi acquire iron from the environment[15]. In the context of endophytism, siderophore production by *A. alternata* could benefit the host plant, especially in calcareous and alkaline soils typical of East Azerbaijan Province, where iron

deficiency is common. This suggests a possible mutualistic role of *A. alternata* in improving host plant nutrition, an aspect that deserves further experimental investigation[16].

The six terpene clusters are likely involved in the production of volatile organic compounds (VOCs). Fungal VOCs play important roles in plant-fungus communication, including promoting plant growth, inducing defense responses, and even repelling herbivores[17]. Therefore, *A. alternata* may contribute to the host plant's resilience not only through direct antagonism of pathogens but also via VOC-mediated signaling.

One of the most intriguing questions raised by our previous study[5] is: how does the high genetic diversity observed among *A. alternata* isolates relate to their biosynthetic capacity? In that study, we detected substantial genetic variation using ISSR markers, with most diversity occurring within populations (72%). However, the present genome mining results suggest that the core BGC repertoire – the set of 42 BGCs – is likely conserved across all isolates, given their phylogenetic proximity to the reference genome.

This apparent contradiction (high neutral genetic diversity but conserved biosynthetic machinery) has been observed in other fungal systems. For example, a recent study on *Alternaria* species from lettuce showed that BGC content was more strongly predicted by phylogeny (i.e., species identity) than by host type or ecological lifestyle[18]. Our findings are consistent with this pattern: *A. alternata* maintains a core set of BGCs regardless of the host plant, implying that these clusters are essential for its endophytic lifestyle. Genetic diversity, therefore, may be concentrated in non-biosynthetic regions (e.g., regulatory genes, transposable elements) or in accessory BGCs that were not captured by our genome-mining approach (since we used a single reference genome). This hypothesis can be tested in future studies by sequencing the genomes of multiple isolates from different hosts and comparing their BGC complements[19,20,21].

Based on the BGCs identified, we propose the following ecological roles for *A. alternata* as an endophyte (Table 2):

Table 2

Proposed ecological roles of major biosynthetic gene cluster (BGC) classes predicted in the *Alternaria alternata* reference genome, and their potential beneficiaries in the endophytic association.

| Metabolite class | BGC type | Putative ecological role | Beneficiary |
|---|---|---|---|
| Alternariol, Altenuene | T1PKS | Antimicrobial, anti-feeding, defense against pathogens | Host plant |
| Tenuazonic acid | NRPS | Condition-dependent pathogenicity or competition | Fungus |
| Fumonisin-like | Hybrid T1PKS-NRPS | Complex metabolite, potential defense | Unknown / both |
| Ferrichrome-like | Siderophore | Iron acquisition, improved host nutrition | Host plant |
| Sesquiterpenes, Erythrenolide | Terpene | Volatile compounds, chemical communication, defense signaling | Plant-fungus interaction |
| Cyclopiazonic acid-like | Indole | Anti-insect, anti-herbivore | Host plant |
| Betalactones | Betalactone | Potential antimicrobial activity | Host plant |

Note: These roles are hypothetical and based on *in silico* predictions. Experimental validation (e.g., gene expression, metabolomics) is required to confirm actual production and activity under endophytic conditions.

It is important to note that the presence of a BGC does not guarantee expression under endophytic conditions. Many BGCs are silent or expressed only under specific environmental triggers (e.g., stress, host signals)[22,23]. Therefore, future metabolomic and transcriptomic studies are needed to determine which of these clusters are active during the endophytic phase.

This study has several limitations that should be acknowledged. First, our genome mining was performed on a single reference genome, not on the genomes of our own isolates. While phylogenetic proximity justifies this approach (as confirmed by ITS1), we cannot rule out intraspecific variation in BGC presence/absence among our isolates[24,25]. Second, the ITS1 region alone (single-direction sequencing) provided sufficient resolution for species identification but did not allow us to examine the ITS2 region or obtain full-length ITS sequences[9]. Third, our conclusions about ecological roles are based on *in silico* predictions and require experimental validation.

Future research should focus on: (i) whole-genome sequencing of representative *A. alternata* isolates from different host plants to assess intraspecific BGC variation; (ii) metabolomic profiling (e.g., LC-MS) of cultures grown under different conditions to detect actual metabolite production; (iii) co-culture experiments with plant pathogens to test antimicrobial activity; and (iv) plant bioassays to evaluate growth-promoting effects, especially regarding siderophore-mediated iron nutrition[26,27].

## 6. Conclusion

In conclusion, this study provides the first integrated analysis of genetic diversity and biosynthetic potential in endophytic *Alternaria alternata* populations from medicinal plants in Iran. We confirmed that all isolates belong to *A. alternata*, and we demonstrated that the species possesses an exceptionally rich repertoire of 42 BGCs, including clusters for antimicrobials, siderophores, terpenes, and toxins. These findings suggest that *A. alternata* is not a passive colonizer but rather an active metabolic partner that may contribute to host plant defense and nutrition. By linking our previous population genetics data with genome mining, we offer a new perspective on the relationship between genetic variation and secondary metabolism in a widespread endophytic fungus. Our study also positions *A. alternata* as a promising candidate for bioprospecting novel bioactive compounds with pharmaceutical and agricultural applications.

## Declarations

**CRediT authorship contribution statement**

**Mostafa Ebadi** is the sole author of this work and contributed to all aspects of the study, including conceptualization, experimental design, investigation, data collection and analysis, visualization, writing the original draft, review, editing, and final approval of the manuscript. All the work was carried out by the single author.

# Declaration of Competing Interest

The author has no conflict of interest to declare.

# Note

These roles are hypothetical and based on *in silico* predictions. Experimental validation (e.g., gene expression, metabolomics) is required to confirm actual production and activity under endophytic conditions.

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The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

# Author Contribution

Mostafa Ebadi is the sole author of this work and contributed to all aspects of the study, including conceptualization, experimental design, investigation, data collection and analysis, visualization, writing the original draft, review, editing, and final approval of the manuscript. All the work was carried out by the single author.


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# Data Availability

The sequence data generated in this study have been deposited in the GenBank database under accession numbers [PZ485020–PZ485022]. The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

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# Figures

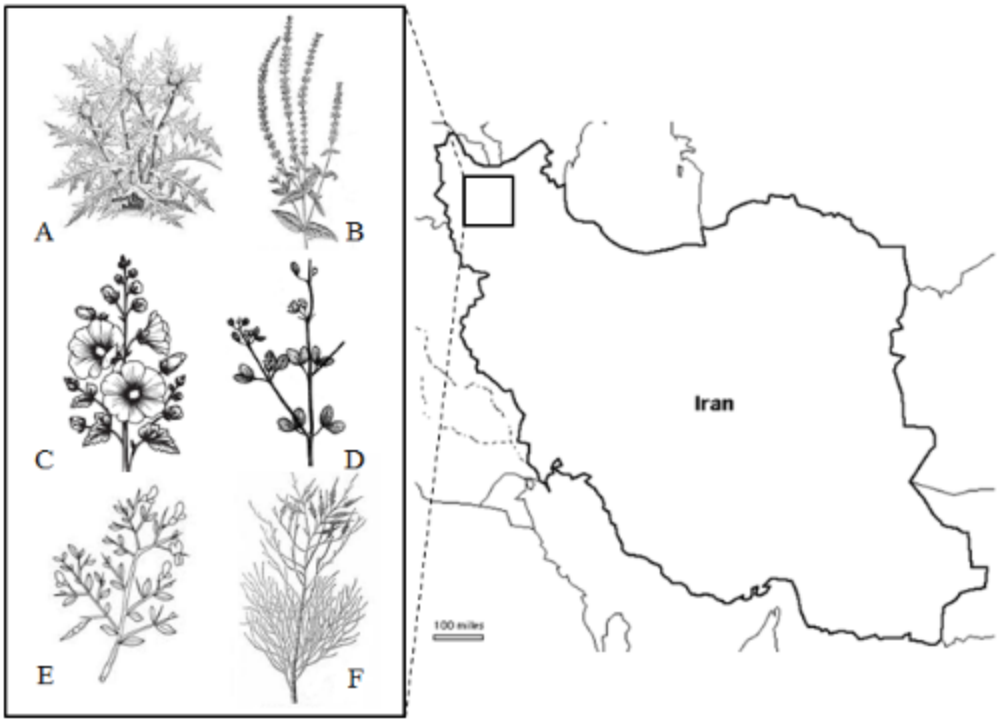


**Figure 1**

Geographic location of sampling site and host plant species. (A–F) Illustrations of the six plant species from which endophytic *Alternaria alternata* isolates were obtained: (A) *Gundelia tournefortii*, (B) *Salvia nemorosa*, (C) *Alcea rosea*, (D) *Zygophyllum fabago,* (E) *Alhagi maurorum*, and (F) *Tamarix ramosissima*. Plant illustrations are schematic representations (adapted from open-source botanical drawings).

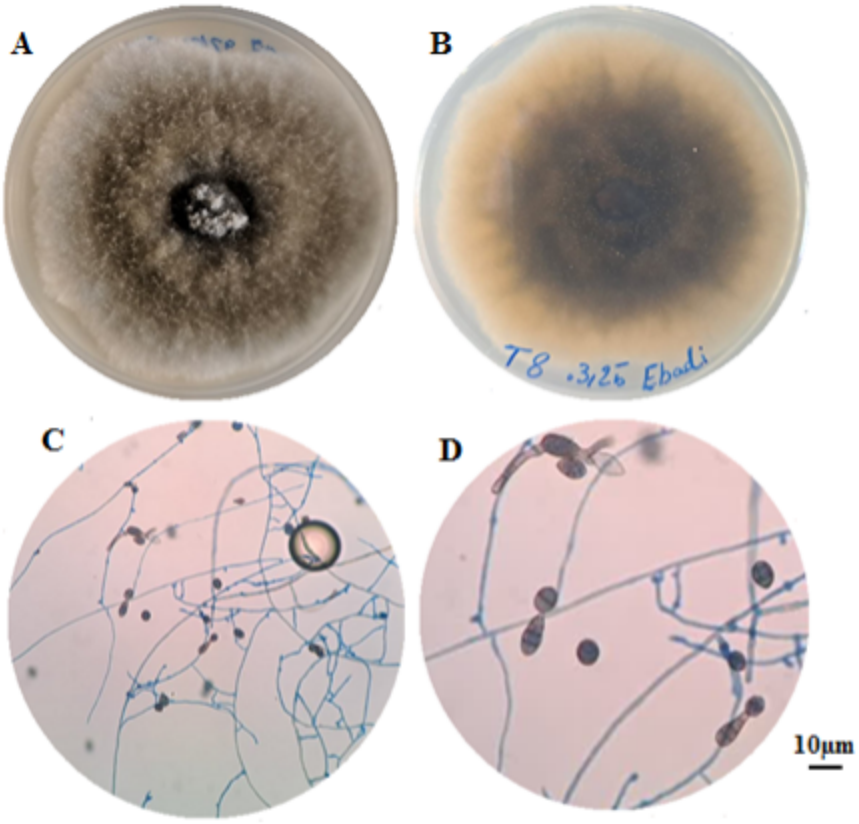


**Figure 2**

Morphology of colony and conidia of *Alternaria alternata* isolates. The colony upper (A) and lower (B) surface, and *A. alternata* mycelium (C) and conidiospores (D).

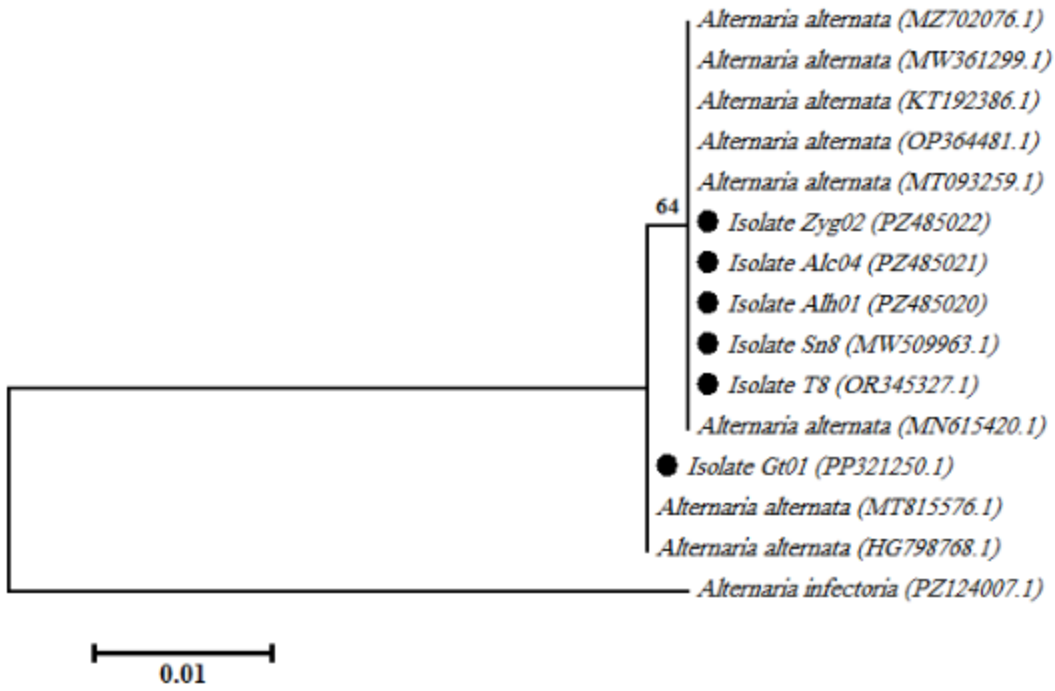


**Figure 3**

Condensed Maximum Likelihood (ML) phylogenetic tree based on ITS1 sequences showing the relationships of endophytic *Alternaria alternata* isolates (●) with reference sequences from GenBank. The tree was constructed using MEGA 5 with the Jukes-Cantor (JC) model. Bootstrap support values (1,000 replicates) are shown at the nodes; values below 50% are not displayed. *Alternaria infectoria* was used as outgroup. The scale bar represents 0.01 substitutions per nucleotide position.

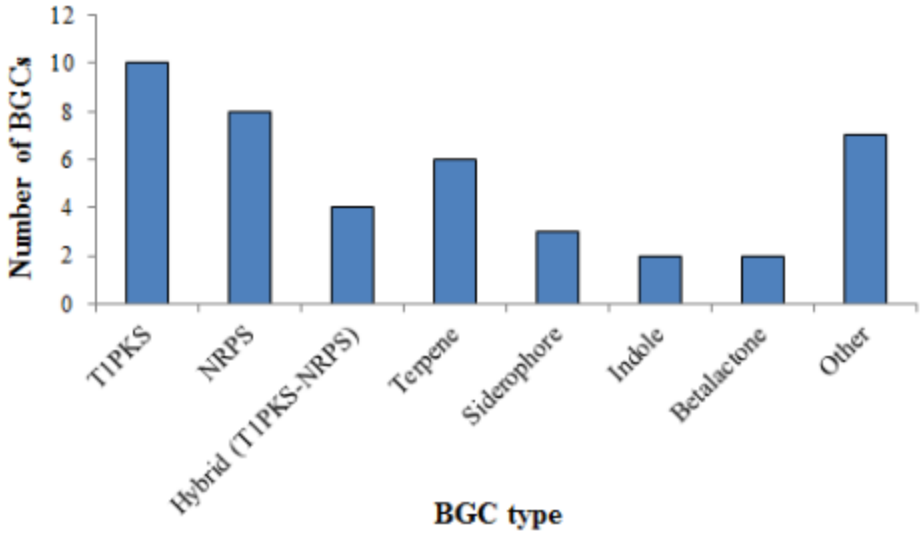


**Figure 4**

Bar chart showing the number and types of biosynthetic gene clusters (BGCs) identified in the *Alternaria alternata* reference genome using antiSMASH v7.1.