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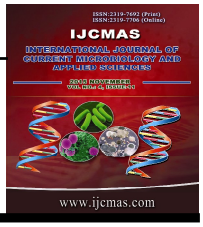


**Original Research Article**

# Antimicrobial Activity of Endophytic Fungi from *Ferula foetida*

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*Ferula foetida*, Endophytic fungi, Antimicrobial activity, Growth stimulators

**A B S T R A C T**

Ten endophytic fungi were isolated from *Ferula foetida* plants which were collected from selected locations in the south-western Kyzyl-Kum, Uzbekistan. The isolates were tested for antimicrobial activity as well as growth stimulators contents. Among selected endophytes, endophytic fungus *Fusarium sambucinum* - *FF59S* possessed most pronounced antimicrobial properties. Growth inhibition zone for *Staphylococcus aureus* was more than 15 mm, similar to reference antibiotic gentamicin. Study of growth stimulating activity showed that all tested endophytes contain both indole acetic and gibberellic acid, while the highest titer of indoleacetic and gibberellic acid was observed in the extracts of *Alternaria sp.*-*FF63L* - 280 μg\ml and 40 μg\ml, and *Fusarium sambucinum-FF59S* - 300 μg\ml and 50 μg\ml, respectively.

## Introduction

Endophytes are microorganisms asymptomatically living in plant tissues and representing a rich source of bioactive metabolites (Owen and Hundley, 2004; Strobel *et al.*, 2004; Tan and Zou, 2001). In accordance with recent evidences, the coexistence of plants and endophytes is mutually beneficial and provides improved viability of both partners (Newmanand and Cragg, 2007). Endophytes can improve plant survivorship rate, producing a variety of beneficial substances. Being isolated and characterized, endophytes possess large potential for use in the industry, agriculture and medicine. (Gunatilaka, 2006; Newmanand and Cragg, 2007). Considering that relatively limited number of endophytes have been discovered, many research groups put substantial effort to identify the potential applicability of these microorganisms in the production of bioactive substances.

Each plant (from nearly 300 000 species inhabiting earth) hosts from one up to several endophytes (Strobel *et al.*, 2003). The diversity of endophytes plays an important part in ecosystems, specifically, in preserving valuable and endangered plant species. Various species of the genus *Ferula* (*Apiaceae*) have a long ethnobotanical

history, since for many centuries these plants were used in biomedical applications. Avicenna applied fetid gum to restore body's defenses and in the treatment of infectious, ocular, neuropsychiatric and skin diseases. Many biological characteristics of this genus as cytotoxicity, antibacterial, antiviral and anti-inflammatory properties are due to a complex composition of bioactive secondary metabolites (as coumarins, phenylpropanoids and sesquiterpenes) contained in the plants (Dall'Acqua *et al.*, 2011; Nazari and Iranshahi, 2011). Considering the diverse biological properties of substances obtained from the genus *Ferula* plants and unprecedented interest in the use of natural products as a new generation of therapeutic agents, these plants' endophytic fungi are of interest as an alternative source of similar bioactive compounds. This paper presents the first data on the fetid gum's endophytic fungi growing on the territory of Uzbekistan.

## Materials and Methods

**Study area and material sampling:** *Ferula foetida* plants were collected in April 2014 on the territory of the south-western KyzylKum (foothills of Mount Kulzhuntog) and preserved at +4°C temperature. Plant samples were identified and stored in a herbarium.

**Isolation of endophytic fungi:** Endophytic fungi were isolated by the method as described previously by Hazalin *et al.* (2009). Stems, leaves and inflorescences of harvested plants were respectively washed in tap water, sterilized in 70% ethanol for 1 min followed by 0.1% $HgCl_2$ for 7 min, rinsed three times in de-ionized water, cut into segments approximately 2-5 mm in diameter and placed in 90 mm Petri dishes containing Czapek-Doxagarized medium with 50 mg/ml chlortetracycline and 250 mg/ml streptomycin sulfate to inhibit bacterial growth. The plates were incubated for 7–14 days at 28°C. Different mycelia growing out of the segments were sub-cultured and individually maintained on antibiotics-free Czapek-Dox-agar medium. Colony morphology and growth and spore formation of the isolates were then studied on Czapek-Dox-agar medium.

**Endophytic fungi identification:** Isolated strains were identified by classical methods on the basis of morphology using pertinent monographs (Litvinov, 1967). Isolated strains were deposited at the Institute of Microbiology of the Uzbekistan Academy of Sciences where they were maintained at low temperature (4–5°C).

**Fermentation and extraction:** To accumulate biomass for further extraction and determination of biological activity, endophytes were grown by submerged fermentation in 500 ml flasks containing 200 ml of Chapek-Dox liquid medium on rotary shaker at 180 rpm and a temperature of $28^0$C for 5 days. The fermentation broth of each endophyte was centrifuged at 6 000 rpm to separate the filtrate from the mycelia.

**The extraction of secondary metabolites of endophytic fungi:** To determine the antibacterial and antifungal activities, metabolites from biomass were extracted by methods as described previously by Lang *et al.* (2005). 5 g of biomass was milled in a Potter homogenizer, transferred to a cone flask containing 50 ml of ethyl acetate, and left for extraction at night on a shaker at room temperature. The mixture was filtered through filter paper (Whatman No. 1) and $Na_2SO_4$ (40 µg/ml) was added. After the filtration, the extract was striped to dryness on a rotary evaporator and mixed with 1 ml of dimethyl sulfoxide. The resulting extract was used as a stock solution and stored at 4°C.

**Antimicrobial assay:** The fungal extracts were screened using the agar diffusion method for antimicrobial activity against potentially pathogenic bacteria *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas* and phytopathogen fungi *F. oxysporum*, *F. solani*, *F. gillosum*, *F. vasinfectum* and *F. senitectum*. Antimicrobial activity was assessed by the size (diameter in mm) of the inhibition zones. Gentamicin sulphate at a concentration of 10 U/ml and nystatin at a concentration of 1 ug/ml were used as standard antibacterial and antifungal agents respectively, while sterilized water was used as the negative control. Each inhibition experiment was repeated three times. Testing cultures were grown on beaf-extract agar for bacteria and Sabouraud's glucose agar for fungi ("HiMedia", India). The Petri dishes with corresponding nutrient solutions were inoculated with daily testing culture suspension in physiological solution with a concentration of $1x10^6$, dried, cut 5 mm diameter wells in agar, embedded with 300 µl of extract and incubated at a temperature of 37°C for 24–48 hours, and fungi at a temperature 28°C for 72–96 hours, respectively.

## Results and Discussion

Ten isolates of endophytic fungi were recovered from the stem (6 isolates) and leaves (4 isolates) of fetid gum (*Ferula foetida*) (Figure 1).

As shown in table 1, the isolated endophytic fungi contained three strains of the *Alternaria genus*, two strains of *Fusarium*, *Aspergillus* and *Ulocladium* genus, and one representative of the *Acremonium* genus.

*Escherichia coli* (EC), *Staphylococcus aureus* (SA), *Pseudomonas aeruginosa* (PA), *F. gillosum* (FG), *F. oxysporum* (FO), *F. solani* (FS), *F. senitectum 1* (FS1), and *F. vasinfectum* (FV) testing cultures (deposited in the Department of Collection of Industrially Important Cultures of Microorganisms of the Institute of Microbiology under the Uzbekistan Academy of Sciences) were used to determine extracts' antimicrobial activity from tested strains' biomass (Table 2).

Table 2 shows that the extracts of five studied endophytes contain metabolites with an extra trace inhibitory activity against some of the used testing cultures. The antibacterial activity, comparable to a standard antibiotic, was detected only in the biomass *F. sambucinum - FF59S* extract, isolated from the fetid gum stem. Growth inhibition zone for *S. aureus* was more than 15 mm, similar to reference antibiotic gentamicin. At the same time, the extract exhibited minimal activity against *E. coli*, and was inactive against *P. aeruginosa*.

It is established that the extract of the same endophyte contains metabolites inhibiting phytopathogens *F. gillosum* and *F. vasinfectum* growth from more than 15 mm. Although the extract growth inhibitory activity is twice lower than reference antibiotic (30 mm), the evidence obtained from the study presents practical value as it indicates on the presence of the active substances.

Considering low concentration of growth inhibiting metabolites in extracts, pathogens' growth was studied with six-fold concentrated extracts. Contrary to our expectations, concentrated extracts of all endophytes except *F. sambucinum - FF59S* was found to enhance testing cultures' growth instead of inhibition (Figure 2).

**Table.1** *Ferula foetida* endophytic fungi composition

| # | Part of a plant | Isolate name |
|---|---|---|
| 1 | Stems | *Fusarium sambucinum - FF59S* |
| 2 | | *Fusarium sp.- FF60S* |
| 3 | | *Alternaria sp. - FF61S* |
| 4 | | *Acremonium coremioides - FF79S* |
| 5 | | *Aspergillus fumigatus - FF80S* |
| 6 | | *Ulocladium sp. - FF82S* |
| 7 | Leaves | *Alternaria sp. - FF62L* |
| 8 | | *Alternaria sp. - FF63L* |
| 9 | | *Ulocladium sp. - FF64L* |
| 10 | | *Aspergillus terreus - FF65L* |

**Table.2** Antimicrobial activity of *Ferula foetida* fungal endophytes

| # | Testing cultures | ***EC*** | ***SA*** | ***PA*** | ***FG*** | ***FO*** | ***FS*** | ***FS1*** | ***FV*** |
|---|---|---|---|---|---|---|---|---|---|
| | Endophytes | Pathogen growth inhibition zone, mm | | | | | | | |
| 1 | *FF59S* | 3 | **15** | - | **15** | 3 | **-** | - | **15** |
| 2 | *FF61S* | - | - | - | - | - | - | - | 2 |
| 3 | *FF80S* | - | - | - | 1 | - | - | - | - |
| 4 | *FF82S* | - | - | - | - | - | 1 | - | - |
| 5 | *FF63L* | - | - | - | - | - | 8 | 1 | - |
| 6 | *FF64L* | 2 | - | - | - | - | 1 | 2 | - |
| 7 | gentamicin | 25 | 15 | 15 | | | | | |
| 8 | nystatin | | | | 30 | 30 | 30 | 30 | 30 |

**Table.3** IAA and GA content in endophytes *Ferula foetida*

| # | Endophytic fungi | GA (µg\ml) | IAA (µg/ml) |
|---|---|---|---|
| 1 | *Fusarium sambucinum-FF59S* | 50 | 300 |
| 2 | *Fusarium sp.- FF60S* | 10 | 50 |
| 3 | *Alternariasp.- FF61S* | 30 | 190 |
| 4 | *Acremonium coremioides- FF79S* | 10 | 200 |
| 5 | *Aspergillus fumigatus - FF80S* | 30 | 210 |
| 6 | *Ulocladium sp. - FF82S* | 20 | 180 |
| 7 | *Alternaria sp.-FF62L* | 30 | 210 |
| 8 | *Alternaria sp.-FF63L* | 40 | 280 |
| 9 | *Ulocladium sp.- FF64L* | 20 | 150 |
| 10 | *Aspergillus terreus- FF65L* | 40 | 250 |

**Figure.1** Fetid gum (*Ferula foetida*) growing in south-west of Kyzyl-Kum (foothills of Kuljuytog mountain)

**Figure.2** Pathogens' growth inhibition zone by extracts of *Fusarium sambucinum* - *FF59S* isolated from fetid gum stem (a) - non concentrated, b) - concentrated)

a)

b)

In this regard, an analysis of the content of growth stimulators (indole acetic and gibberellic acid) in the metabolites of isolated endophytes was implemented. The findings show that all tested endophytes contain IAA and GA (Table 3). It should be noted that growth stimulating activity is typical for many endophytes. However, endophytes *Ferula foetida* contain much higher rate of IAA and GA as compared to previously studied endophytes (Khan *et al.*, 2013).

Table 3 reveals that the extracts of *Alternaria sp.-FF63L* and *F. sambucinum-FF59S*, isolated from the leaves and stem of fetid gum, contain the highest number of both IAA and GA.

In conclusion, the results from the study show that fetid gum is inhabited by endophytic fungi that have antimicrobial and growth stimulating activity. Of 10 selected endophytes, endophytic fungus *F. sambucinum-FF59S* possesses most pronounced antimicrobial and growth stimulating properties. The last finding provides promising avenue for use it in biotechnological applications.

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