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ANTIMICROBIAL SCREENING AND MOLECULAR IDENTIFICATION OF ACTIVE ENDOPHYTIC BACTERIAL ISOLATES FROM *FERULA VARIA*

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Abstract: Endophytic bacteria from medicinal plants are a promising source of antimicrobial metabolites, and this study evaluated the endophytic isolates of Ferula varia (more than 40 isolates from roots, stems, leaves, and flowers) against Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtilis, and Candida albicans. Six isolates showed antimicrobial activity and were identified by 16S rRNA gene sequencing as Bacillus pumilus, B. atrophaeus, B. rugosus, and Serratia odorifera, with the sequences deposited in NCBI GenBank. Four of these isolates (FV1i, FV3i, FVP1.1, and FVB1) were active against all five test organisms, making them promising candidates for further study of their bioactive metabolites.

Keywords: *Ferula varia*, endophytic bacteria, antimicrobial activity, inhibition zone, 16S rRNA gene sequencing, molecular identification, antimicrobial metabolites.

Introduction

Endophytic bacteria are microorganisms that live within the internal tissues of plants, usually without causing disease symptoms in the host plant, and are regarded as a promising source of biologically active metabolites. Medicinal plants play an important role in studying the diversity of endophytic microorganisms and in the search for new antimicrobial agents. Studying the antimicrobial properties of endophytic bacteria associated with *Ferula varia* makes it possible to identify biotechnologically significant microorganisms. The aim of this study was to evaluate the antimicrobial activity of endophytic bacteria isolated from *F. varia* against selected bacterial and fungal test organisms and to identify the active isolates at the molecular level using 16S rRNA gene sequencing.

Materials and Methods

In the primary screening, more than 40 endophytic bacterial isolates obtained from the roots, stems, leaves, and flowers of *F. varia* were tested for antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Candida albicans*. Antimicrobial activity was determined by measuring the diameters of the growth inhibition zones of the test organisms. The active isolates were identified at the molecular level by 16S rRNA gene sequencing, and the resulting nucleotide sequences were analyzed to determine the taxonomic affiliation of the isolates.

Results and Discussion

Among the endophytic bacteria isolated from *F. varia*, isolates FV1i, FV3i, FV8i, FVP2, FVP1.1, and FVB1 exhibited antimicrobial activity against the test organisms (Table 1) and were selected for further studies.

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Table 1. Antimicrobial activity of the isolates (diameter of the growth inhibition zone)

Isolates	S. aureus (mm)	Ps. aeruginosa (mm)	B. subtilis (mm)	E. coli (mm)	C. albicans (mm)
FV1i	20	28	20	29	16
FV3i	21	29	26	33	16
FV8i	–	–	–	12	–
FVP2	–	–	12	18	–
FVP1.1	33	12	14	12	12
FVB1	27	14	18	28	14

The selected isolates were identified at the molecular level using the 16S rRNA gene marker. According to the identification results, five of the six selected isolates belonged to the genus *Bacillus* and one to the genus *Serratia*. The resulting nucleotide sequences were deposited in the NCBI GenBank database under the accession numbers listed in Table 2.

Table 2. Results of the molecular identification of the isolates based on the 16S rRNA gene

Isolated Strains Sequences Deposited in GenBank		Closest Match among Bacteria (16S rRNA Genes) (GenBank)		
Strains	**Accession Number**	**Species**	**Source**	**ID%**
FV1i	PZ582851	*Bacillus pumilus*	*F. varia*	100%
FV3i	PZ583085	*Bacillus atrophaeus*	*F. varia*	100%
FV8i	PZ583143	*Bacillus atrophaeus*	*F. varia*	99,86%
FVP2	PZ583123	*Bacillus rugosus*	*F. varia*	99,38%
FVP1.1	PZ054243	*Bacillus rugosus*	*F. varia*	99,86%
FVB1	PZ331847	*Serratia odorifera*	*F. varia*	99,93%

Conclusion

In this study, six active isolates of endophytic bacteria associated with *F. varia* were investigated with respect to their antimicrobial activity and molecular identification. The activity of the isolates against the test organisms *E. coli*, *S. aureus*, *P. aeruginosa*, *B. subtilis*, and *C. albicans* was evaluated, and 16S rRNA gene sequencing showed that five of them belonged to the genus Bacillus and one to the genus *Serratia*. These data provide a basis for the further characterization of these isolates, a more detailed investigation of their antimicrobial potential, and the identification of their biologically active metabolites.

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