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ANTIBACTERIAL POTENTIAL OF THE ENDOPHYTIC FUNGAL ISOLATE S34 FROM *THERMOPSIS ALTERNIFLORA*

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Abstract: The ethyl acetate extract of the endophytic fungal isolate S34, obtained from *Thermopsis alterniflora* collected in Bostanliq District, Tashkent Region, was screened for antimicrobial activity by the agar disk-diffusion method. The extract inhibited *Bacillus subtilis* (12 ± 0.33 mm), *Pseudomonas aeruginosa* (9 ± 0.19 mm) and *Staphylococcus aureus* (7 ± 0.12 mm), whereas no activity was detected against *Escherichia coli* or *Candida albicans*.
Keywords: endophytic fungi, *Thermopsis alterniflora*, antimicrobial activity, disk diffusion, *Pseudomonas aeruginosa*.

Annotatsiya: Toshkent viloyati Bo‘stonliq tumanidan yig‘ilgan *Thermopsis alterniflora* o‘simligidan ajratilgan endofit zamburug‘ S34 izolyati etilatsetatli ekstraktining antimikrob faolligi agar-disk diffuziya usulida baholandi. Ekstrakt *Bacillus subtilis* (12 ± 0,33 mm), *Pseudomonas aeruginosa* (9 ± 0,19 mm) va *Staphylococcus aureus* (7 ± 0,12 mm) o‘sishini to‘xtatdi, *Escherichia coli* va *Candida albicans*ga nisbatan esa faollik kuzatilmadi.
Kalit so‘zlar: endofit zamburug‘lar, *Thermopsis alterniflora*, antimikrob faollik, disk-diffuziya, *Pseudomonas aeruginosa*.

Аннотация: Методом диффузии в агар (диско-диффузионный метод) оценена антимикробная активность этилацетатного экстракта эндофитного гриба S34, выделенного из растения *Thermopsis alterniflora*, собранного в Бостанлыкском районе Ташкентской области. Экстракт подавлял рост *Bacillus subtilis* (12 ± 0,33 мм), *Pseudomonas aeruginosa* (9 ± 0,19 мм) и *Staphylococcus aureus* (7 ± 0,12 мм), тогда как в отношении *Escherichia coli* и *Candida albicans* активность не выявлена.
Ключевые слова: эндофитные грибы, *Thermopsis alterniflora*, антимикробная активность, диско-диффузионный метод, *Pseudomonas aeruginosa*.

Introduction

Antimicrobial resistance has become one of the most serious threats to global health: in 2019 alone, an estimated 1.27 million deaths were directly attributable to resistant bacterial infections [1]. This situation creates an urgent need for new sources of antimicrobial compounds. Endophytic fungi, which colonize internal plant tissues without causing visible symptoms, are widely regarded as a prolific source of structurally diverse and biologically active secondary metabolites [2]. Plants of the genus *Thermopsis* (Fabaceae) are rich in quinolizidine alkaloids, and cytisine has been quantified in *T. alterniflora* [3]; however, the endophytic fungi inhabiting this plant have received little attention. The aim of this study was to evaluate the antimicrobial activity of the extract of the endophytic fungal isolate S34 obtained from *T. alterniflora*.

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Materials and methods

Plant material of *T. alterniflora* was collected in Bostanliq District, Tashkent Region, Uzbekistan, and isolate S34 was recovered from surface-sterilized plant tissues. The isolate was cultivated in potato dextrose broth for 21 days, after which the culture was extracted with ethyl acetate. The antimicrobial activity of the extract (20 mg/mL) was determined by the agar disk-diffusion method as described previously [4, 5] against Gram-positive (*Bacillus subtilis*, *Staphylococcus aureus*) and Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*) bacteria and the yeast *Candida albicans*. Ampicillin/sulbactam, gentamicin and fluconazole served as positive controls for Gram-positive bacteria, Gram-negative bacteria and *C. albicans*, respectively. All assays were performed in triplicate, and the results are expressed as mean ± standard error (SE).

Results

The S34 extract was active against three of the five test organisms. The most sensitive strain was *B. subtilis*, with an inhibition zone of 12 ± 0.33 mm, which amounts to 40.0% of the zone produced by ampicillin/sulbactam (30 ± 0.21 mm). Against the Gram-negative *P. aeruginosa*, the extract formed a zone of 9 ± 0.19 mm, equal to 34.6% of the gentamicin effect (26 ± 0.41 mm). Only weak inhibition was observed for *S. aureus* (7 ± 0.12 mm; 26.9% of the ampicillin/sulbactam zone, 26 ± 0.28 mm), while *E. coli* and *C. albicans* were not susceptible to the extract.

Discussion

The activity of the extract against *P. aeruginosa* deserves particular attention, since this species is intrinsically resistant to many antibiotics owing to the low permeability of its outer membrane and its efficient efflux systems [6]. Anti-*Pseudomonas* activity has also been recorded for the ethyl acetate extract of *Alternaria alstroemeriae* S6, an endophyte of *Veronica acinifolia* previously studied by our group [4]. At the same time, the S34 extract did not affect the other Gram-negative species, *E. coli*, which may point to a species-selective mode of action of its active constituents; this assumption, however, requires further verification. Because the disk-diffusion assay is a qualitative screening method and the zone diameter also depends on how well compounds diffuse through agar [5], minimum inhibitory concentrations (MIC) must be determined for a quantitative assessment. In addition, the present data were obtained with a crude extract, and the metabolites responsible for the activity have not yet been identified.

Conclusion

The endophytic fungal isolate S34 from *T. alterniflora* produces metabolites that inhibit both Gram-positive and Gram-negative bacteria, including *P. aeruginosa*. Further work will include molecular identification of the isolate, determination of MIC values, and bioassay-guided fractionation of the extract followed by chemical characterization of the active compounds.

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