Biocontrol and Plant Growth Promoting Potential of Strain *Streptomyces* sp. A537 isolated form *Suaeda salsa* non-rhizosphere soil

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Abstract. *Fusarium oxysporum* is a serious pathogenic fungus that can cause peanut root rot, leading to reduced peanut yield, plant death, and severely hindering the development of agriculture and economy in peanut cultivation region. This study isolated a large number of bacterial strains from soil samples of wild plants such as *Suaeda salsa*, *Tamarix*, and *Phragmites australis* in the Aydingkol Lake area of Xinjiang, and evaluated their biocontrol and capability of promote plant growth. Finally, an actinomycete strain A537 was isolated and screened from the non-rhizosphere soil of *Suaeda salsa*. The sequencing results of 16s RNA indicate that A537 belongs to the genus *Streptomyces*. The plate confrontation assay showed that strain A537 has a wide antifungal spectrum and has certain antagonistic activities to various plant pathogens such as *Fusarium oxysporum*, *Fusarium solani*, *Diplodia gossypina*, *Fusarium equiseti*, *Fusarium acuminatum*, *Verticillium dahlia*, *Rhizoctonia cerealis*, *Fusarium pseudograminearum* and *Fusarium graminearum*. In addition, in vitro evaluation of PGP traits showed that strain A537 has the capability of phosphate solubilization, IAA production, siderophore production, ACC deaminase production and cellulases production. Different concentrations of culture filtrate of strain A537 have a certain antagonistic activities on *Fusarium oxysporum*, with an inhibition rate of 76.54 ± 6.79%. The results indicate that strain *Streptomyces* sp. A537 has the potential for biological control and growth promotion, and can be used as a novel biocontrol agent against peanut root rot.

1 Introduction

Peanuts (Arachis hypogaea L.) is an important oil crop and economic crop, as well as also one of the important raw materials for the world food industry. It is widely planted worldwide [1]. Peanut root rot is a serious soil-borne disease caused by fungi of *Fusarium* spp., mainly including *Fusarium oxysporum* and *Fusarium solani* [2, 3]. *Fusarium oxysporum* can infect peanuts throughout their entire growth period, mainly causing seedling decay, vascular wilt, browning of main roots, and reduction of lateral roots, ultimately leading to plant withering and death[4, 5]. In recent years, with the large-scale and consecutive years of cropping of peanuts, the incidence of peanut root rot disease has been increasing, leading to slow growth and decreased yield, seriously threatening the agricultural and economic development of peanut cultivation regions [6-8]. Therefore, it is crucial to find scientifically safe and effective prevention and control methods.

Currently, the prevention and control measures for peanut root rot mainly include application of cultivation measures, chemical control, and biological control. However, cultivation measures such as crop rotation and field cultivation management require a long time and incur certain costs; Chemical control may leave pesticide residues in the fields, causing secondary pollution to the environment. Therefore, green, safe, and environmentally friendly biological control measures have received widespread attention from people. Recent studies have shown that the use of plant growth-promoting rhizobacteria (PGPR) for the prevention and control of plant diseases, which can promote plant growth and achieve sustainable crop development, is increasingly welcomed by people[9].

PGPR refers to a class of beneficial rhizosphere bacteria that can help plants resist plant pathogens and promote plant growth through direct and indirect mechanisms[10]. PGPR can help plants resist the invasion of pathogens in the following ways: (1) synthesis and production of antibiotics; (2) production of generate metabolites with antifungal activity, such as hydrogen cyanide; (3) Production enzymes that can lyse some fungal cells, such as chitinase and cellulases; (4) antagonistic activities to pathogenic fungus; (5) PGPR-mediated induced systemic resistance (ISR), and other methods to reduce the occurrence or severity of plant diseases [11-14]. PGPR has a wide range of members, mainly including *Actinomycetota, Pseudomonadota, Bacillota, Ascomycota*, etc. Among them, *Streptomyces* is one of its representative members [15-19] *Streptomyces*

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has attracted extensive research due to its capability to produce numerous metabolites, and many strains of Streptomyces have been developed into commercial products [20].

Xinjiang is located in the northwest region of China, and due to its vast area and unique environment, it has a large amount of undeveloped microbial resources. In recent years, a large number of researchers have isolated many novel microorganisms with unique functions from Xinjiang [21-23]. Aydingkol Lake is a salt lake in Xinjiang, and due to its unique extreme environment, its soil microorganisms may become effective new biological control agents.

In this study, a strain, *Streptomyces* sp. A537, was isolated form *Suaeda salsa* non-rhizosphere soil. We studied its inhibitory effect of various plant pathogens, investigated its antagonistic activities against *Fusarium oxysporum* at different concentrations of culture filtrate, and evaluated its PGP traits. This study aims to provide a theoretical basis for applying PGPR in peanut root rot.

2 Materials and methods

2.1 Isolation and screening of antifungal bacteria

Soil samples were collected from the rhizosphere and non-rhizosphere of *Suaeda salsa*, *Tamarix*, and *Phragmites australis* in the Aydingkol Lake area of Xinjiang. The soil was naturally died. Bacterias were isolated using the dilution plating method. Single strain colonies were chosen according to their colony size or colour traits on Gao's medium No.1 (GM) plates. Cultivate the purified single colonies three times on a GM plates and stored in 20% glycerol at −80 °C.

The antifungal activity of isolated strains were tested *in vitro* using a dual culturing method. A 8 mm mycelial plug of fungal pathogens was placed at the center of potato-dextrose-agar (PDA) plate, and 20 μL cultures of the strains was added on PDA plate 2.5cm away from the center. After incubation at 28°C for 7 days, the antifungal activity was checked by measuring the diameter of the mycelium.

2.2 Identification of strain A537

The strain A537 colony was grown on GM plates at 28°C for 7 days and observed its morphological characteristics.

The 16S rRNA of strain A537 was amplified by PCR using the following primers: 27F (5' - AGAGTTTGATCTGGCTCAG-3') and 1492R (5' - GGTTACCTTGTTACGACTT-3'), and sequenced. The obtained sequence was analyzed using the BLAST program in NCBI. A phylogenetic tree of the strain A537 was constructed by using the neighbor-joining (NJ) method of MEGA 11.0 software.

2.3 Antagonistic activities of culture filtrates of strain A537

Strain A537 was cultured in potato dextrose broth (PDB) and GM at 28°C for 7 days. The fermentation broth was centrifuged at 6000 ×g for 10 min at 4 °C and filtered through 0.22 μm microfilters to obtain the culture filtrate. The different volumes of culture filtrates were mixed with molten PDA to prepare plates containing culture filtrates with concentrations of 10%, 25%, and 50% (v/v). PDA plates without culture filtrate were used as controls. A 8 mm mycelial plug of *Fusarium oxysporum* were placed at the center of PDA plates and cultured at 28 °C for 7 days.

2.4 Assessment of PGP traits of strain A537

Starin A537 were evaluated for the phosphate solubilization, IAA production, Siderophore production, ACC deaminase production, cellulases production, Nitrogen fixation using method described by [24-26].

3 Experimental Results

3.1 Isolation and identification of strain A537

We isolated a total of 232 strains from soil samples of wild plants such as *Suaeda salsa*, *Tamarix*, and *Phragmites australis* in the Aydingkol Lake area of Xinjiang. One of these isolates, strain A537, showed good antagonistic potential against *Fusarium oxysporum* and was selected for further analysis. Colonies of strain A537 displayed a creamy white, slightly transparent, well-defined margins and the middle was slightly raised.

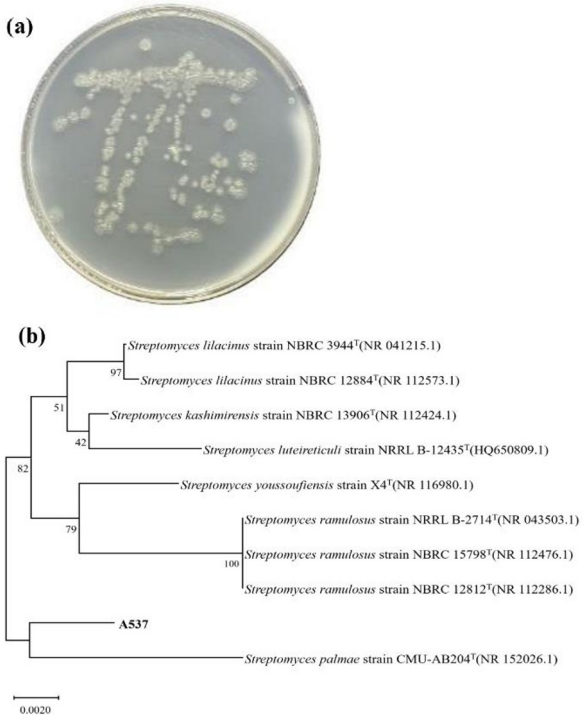

Fig. 1. (a). Morphologic observation of strain A537 (b). Phylogenetic tree derived from neighbor-joining analysis based on a partial 16 S rRNA gene sequence. The number on the branch point represents the credibility Bootstrap values were estimated from 1000 replicate analyses.

Phylogenetic analysis showed that the 16 S rRNA gene sequence of strain A537 clustered closely with species of *Streptomyces*, with a homology of 98.61% with *Streptomyces lilacinus* strain NBRC 3944 (NR_041215.1), indicating that strain A537 may be a potential new species of the genus *Streptomyces*. Based on morphological traits and 16 S rRNA gene sequencing, strain A537 was identified as the member of the genus *Streptomyces* (Fig. 1).

3.2 Antifungal activity of strain A537

3.2.1 Antagonistic activities and inhibition spectrum of strain A537

To evaluate the antifungal spectrum of strain A537, *in vitro* antifungal activity against 10 different fungal pathogens was tested. Strain A537 has a strong inhibitory effect on the growth of 9 of the tested pathogenic fungi(Table 1). Among them, he inhibition rate of strain A537 on *Fusarium oxysporum* was 36.31±4.48%(Fig. 2).

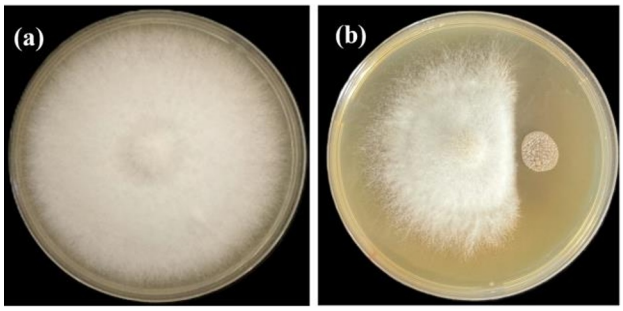

Fig. 2. The Inhibitory effect of strain A537 against *Fusarium oxysporum* (a). Control (b). Strain A537 was pair cultured with *F. oxysporum.*

Table 1. Antifungal spectrum of strain A537.

Plant pathogenic fungi	Results
Fusarium oxysporum	+
Fusarium graminearum.	+
Fusarium pseudograminearum	+
Rhizoctonia cerealis	+
Verticillium dahlia	+
Fusarium acuminatum	+
Fusarium equiseti	+
Diplodia gossypina	+
Fusarium solani	+
Sclerotium rolfsii	-

Note: "+" indicates inhibitory effect, "-" indicates no inhibitory effect.

3.2.2 Antagonistic activities of culture filtrates of strain A537

To test the inhibitory effect of strain A537 on *Fusarium oxysporum*, its culture filtrate was prepared on PDB and GM and used for inhibition test. The results showed that the culture filtrate of strain A537 could inhibit the growth of *Fusarium oxysporum* in both medium, and had a dose-dependent effect(Fig. 3). Among them, the inhibitory effect of PDB culture filtrate is better than that of GM, At a 50% concentration, the inhibition rate was up to 76.54±6.79%(Table 2).

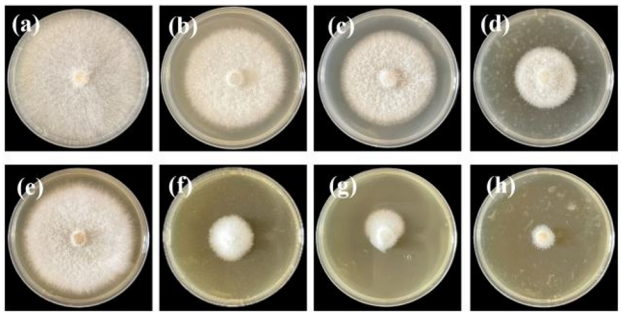

Fig. 3. The Inhibitory effect of two medium culture filtrates of strain A537 against *Fusarium oxysporum* (a-d). GM culture filtrate (b)PDB. culture filtrate (a, e).Control (b,f). Filtrate Concentration 10% (c, g). Filtrate Concentration 25% (d, h)Filtrate Concentration 50%.

Table 2. Inhibitory activity of culture filtrates of two medium culture filtrates of strain A537.

Medium	Filtrate Concentration	Inhibition rate (%)
PDB	10%	11.06±7.26
	25%	20.20±7.48
	50%	42.84±4.86
GM	10%	59.80±0.22
	25%	61.57±15.43
	50%	76.54±6.79

3.3 Plant growth promoting traits of strain A537

Table 3. *In vitro* assessment of PGP traits of strain A537.

PGP traits	Result
Inorganic phosphate solubilization	+
Organic phosphate solubilizatio	+
Siderophore production	+
cellulases production.	+
IAA production	+
ACC deaminase production	+
Nitrogen fixation	-

Note: "+" indicates funcational , "-" indicates non funcational.

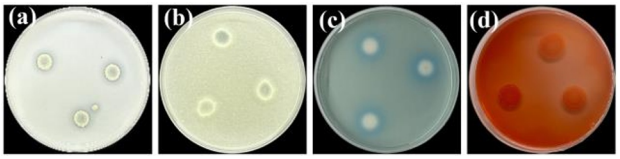

Fig. 4. Determination results of PGP traits of strain A537 (a). Inorganic phosphate solubilization (b). Organic phosphate solubilizatio (c). Siderophore production (d). cellulases production.

In order to evaluate the potential of promoting growth capability of the strain, a series of PGP traits of strain A537 were test in vitro (Table 3), and the results showed that has the capability of phosphate solubilization, IAA production, siderophore production, ACC deaminase

production and cellulases production(Fig. 4). These results indicated that strain A537 has the potential to promote plant growth.

4 Conclusion

In this study, a new strain *Streptomyces* sp. A537 was screened from the non-rhizosphere soil of the wild plant *Suaeda salsa* in Aydingkol Lake, Xinjiang. The results showed that the strain A537 has antagonistic effects on various plant pathogenic fungi. Further research has found that strain A537 has a certain inhibitory effect on *Fusarium oxysporum* in the culture filtrates of both GM and PDB, with PDB culture filtrates showing an inhibitory effect of up to 76.54±6.79%, indicating its potential as a biocontrol agent in the future. In addition, *in vitro* PGP traits test showed that strain A537 possesses multiple PGP traits and has the potential to promote plant growth. This study lays the foundation for further understanding the mechanism of biocontrol of *Fusarium oxysporum* in the future, and offers valuable microbial resources for biocontrol of peanut root rot. In order to accelerate the application process of strain A537, further studies is needed to extract and identify active antifungal substances, and verify strain A537 effectiveness in field conditions.

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