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ISOLATION AND BIOACTIVITY ASSAY OF ACTINOMYCETESIN RHIZOSPHERE OF PAEONIAJISHANENSIS

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ABSTRACT

Actinomyceteis a kind of microbial resources with great medicinal value. Plant rhizosphere soils act as important sites for the survival of actinomycetes. Actinomycetes can help protect the root of plants from pathogenic bacteria infection and some actinomycetes have the ability to produce enzymes and antimicrobial compounds. We studied the antagonistic and enzyme-producing activities of actinomycetes isolated from rhizosphere soil of PaeoniaJishanensis in Yuncheng City, China, so as to provide the scientific basis for the utilization and protection of this endangered plant species. About 52actinomycete isolates were isolated usingserial dilution and spread-plate techniques. The antagonistic activity of actinomycete isolates was assayed using an agar block method against 4 typical bacterial and fungal species. The protease and amylase activities were detected by the transparent plate method. The results show that 11isolates showed antibacterial activity and 20 isolates showed protease and amylaseactivity. In conclusion, rhizosphere soil of PaeoniaJishanensis possesses a high diversity of actinomycetes and serves as a natural source of bioactive compound-producing actinomycetes.

Keywords: Paeoniajishanensis; actinomycetes; antibacterial activity;protease; amylase

1. INTRODUCTION

Actinomycetes are kind of microbial resources with important application value and economic value (Berdy, 2005), which can be used for producing various bioactive substances such as antibiotics, vitamins, enzymes and enzyme inhibitors. It is reported that approximately 45% of bioactive microbial metabolites are produced by actinomycetes(Berdy, 2005). Studies have shown that actinomyces with biological activities in rhizosphere of medicinal plants are significantly higher than those of other plants, due to long-term influences of the medicinal ingredients(Zhang et al., 2016; Guoet al., 2017; Wanget al., 2016; Hinsinger and Marschner, 2006; Schendy, 2016; Caoet al., 2014; Wuet al., 2017; Miyanaga et al., 2006).

PaeconiaJishanensis, a perennial deciduous shrub of *Paeoniaceae*, is an endangered plant endemic to Shanxi Province, China. It is only distributed in narrow area, the southern end of Lvliang Mountain and the western end of Zhongtiaoshan Mountain in Shanxi Province, China, with a small population. It is at risk of extinction at any time (Ji, 2011). It is mainly growing in the middle and low mountain regions, and the altitude is generally between 850 meters and 1,550 meters. The growing of this peony needs good thermal conditions, and it is usually distributed in

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single or dozens of individual plants, and also distributed in clumps or small patches. The height of*PaeconiaJishanensis* usually between 0.4 and 1.0 meters in height, some plants can reach up to 1 meter. The root of *PaeconiaJishanensis* is straight, which can stretch in the crack of rocks. Leaves of *PaeconiaJishanensis* are two pinnate compound leaves, germinates in late march every year, little leaf can amount to 15. The flowering period is late April to mid-May.It is precious Chinese herbal medicine resource and flower resource. The root-bark (danpi) of PaeconiaJishanensis can be used medicinally. For a long time, the clinical use of danpi in Shanxi and Shaanxi almost comes from wild peony, and PaeconiaJishanensis is the biggest victim (Zhang, 2003).

We suggest that the rhizosphere of *PaeconiaJishanensis* living abundant actinomyces, which are closely related to the growth, development, reproduction and metabolic activities of the plants. In recent years, there have several reports on*PaeconiaJishanensis*. These researchesarefocused on its distribution area, growth law and elements in the soil and habitat(Zhou et al., 2013; Coombs and Franco, 2003). However, no research has specifically analyzed the characteristics of actinomycetes in the rhizosphere of *PaeconiaJishanensis*.

To this end, this study investigated the bioactivities of actinomycetes residing in the rhizosphere of *PaeconiaJishanensis*. We evaluated the potential for isolating novel bioactive compound-producing actinomycetes from the special ecosystem of rhizosphere by isolation of actinomycetes and evaluation of their antagonistic, protease and amylase activities. The results were analyzed to explore the distribution of bioactiveactinomycetes in the rhizosphere of *PaeconiaJishanensis* so as to facilitate actinomycete screening for novel bioactive-compound producers from natural resources. In addition, the results are benefit for the protection and utilization of endangered plant species.

2. MATERIALS AND METHODS

Collection of samples

Samples were collected from Yuncheng City,ShanxiProvince, China.The root of *PaeconiaJishanensis*under 10 cm soil was collected, put into sterilized bags, took back to the lab immediately and stored at 4 ℃ for use.

Isolation of actinomycetes

Serial dilution and spread-plate techniques (Yao, 2007) were used to isolate actinomycetes from rhizosphere soil samples. Serial dilutions were made by adding 10 g of root with soil to 50 mL of sterile distilled water (10^{-1}) in a conical flask, followed by oscillation at 160 rpm for 10 min and further dilution to 10^{-5}. The dilutions of 10^{-3} to 10^{-5} (0.05 mL aliquots) were inoculated to the agar media by spread plating.

Five agar media were tested: Gause's synthetic agar (GS, soluble starch 20.0 g; KNO_3 1.0 g; K_2HPO_4 0.5 g; $MgSO_4 \cdot 7H_2O$ 0.5 g; NaCl 0.5 g; $FeSO_4$ 0.01 g; agar 10.0 g; distilled water 1000 mL), modified humic acid agar (HV, humic acid 10.0 g; Na_2HPO_4 0.5 g; KCl 1.0 g;

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$MgSO_4 \cdot 7H_2O$ 0.05 g; $CaCl_2$ 1.0 g; agar 10.0 g; distilled water: 1000 mL), ISP2 (yeast extract 4.0 g; malt extract 10.0 g; glucose 4.0 g; agar 10.0 g; distilled water: 1000 mL), TWYE (yeast extract 0.25 g; K_2HPO_4 0.5 g; agar 10.0 g; distilled water: 1000 mL) and beef extract peptone agar (BPA, beef extract3.0 g; peptone10 g; NaCl5.0 g; agar 10.0 g; distilled water: 1000 mL). All media were supplemented with 80 mg/L potassium dichromate to inhibit the growth of bacteria and fungi(Si et al., 2004). After inoculation, all plates were incubated at 28℃ for 15 days.

Actinobacterial colonies were identified by visual examination of the cultural and morphological characteristics; microscopic examination was performed if needed. Morphologically distinct colonies were transferred onto Gause's synthetic agar slants separately, incubated at 28℃ for 7 days, and then stored in the dark at 4°C. All experiments were performed in triplicate.

Antimicrobial Activity Assay

Antimicrobial activity of actinobacteria isolates was analyzed using an agar block method against four typical species, *Escherichia coli* E1, *Staphylococcus aureus* S4, *Penicillium* sp. P1 and *Saccharomyces cerevisiae* C1,provided by the Microbiology Laboratory in Shanxi Normal University.

Enzymatic activity test

The protease and amylase activities of isolates were detected by the transparent plate methodby adding 10 g/L soluble starch or 250 ml/L skim milk to the basic medium(Zhanget al., 2014).The basic medium was H1 agar (glucose 10.0 g;peptone 5.0 g;yeast extract 5.0 g;$MgSO_4 \cdot 7H_2O$ 0.2 g;$K_2HPO_4$1.0 g;agar 10.0 g; distilled water: 1000 mL). When detecting the protease activity, the transparent was directly observed. When detecting amylase activity, 5 mL of iodine solution was added to cover the plate until clear transparent appeared.

3.RESULTS

Actinomycete isolate numbers

By serial dilution and spread-plate techniques, 82 actinomycetes were isolated from rhizosphere of *PaeconiaJishanensis*, and 52 strains were obtained after removing the repeats.

Table 1 Isolate numbers on five isolation media

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Items	GS	TWYE	ISP2	HV	BPA
Isolate numbers	33	27	7	10	5
Ratio	63.5%	51.9%	13.5%	19.2%	9.6%

The numbers of isolates were varied in different isolation media. On Gause's synthetic agar, 33 isolates (63.5%) were isolated, which was much higher than those of other media. The following medium was TWYE. Only 5 isolates were gain on BPA, the least among all media (Table 1). Colony morphology of 30 isolates was shown in Fig 1.

Fig 1. Colony morphology of 30 isolates

Antimicrobial activity

The antimicrobial activity of 52 actinomycetes isolates was detected using 4 representative target microorganisms. The results showed that 52 isolates presented different antibacterial activity (Table 2).

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A total of 11 isolates showed antagonistic activity. Among that, 2 isolates were antagonistic to *Saccharomyces cerevisiae*, 6 isolates were antagonistic to*Penicillium*sp., 3 isolates were antagonistic to *Staphylococcus aureus*, while no isolate was antagonistic to*Escherichia coli* and no isolate was antagonistic to two or more target micoorganisms (Table 2).

Additionally, isolate g26-3 showed strongest antagonism to *Saccharomyces cerevisiae*, isolate G20-2 showed strongest antagonism to*Penicillium*sp., and isolate G7 showed strongest antagonism to *Staphylococcus aureus*(Table 2).

Table 2 Antimicrobial activities of actinomycete isolates

Target microorganisms	Isolates	Diameter of agar block (cm)	Diameter of transparent (cm)	Ratio*
Saccharomyces cerevisiae	G26-3	0.6	1.4	2.3
	J7	0.6	1.2	2.0
*Penicillium*sp.	G10	0.6	1.1	1.8
	G20-2	0.6	1.3	2.2
	G33	0.6	1.1	1.8
	T4	0.6	0.8	1.3
	N4	0.6	1.0	1.7
	J3	0.6	1.2	2.0
Staphylococcus aureus	G7	0.6	2.1	3.5
	G16	0.6	1.1	1.8
	G28	0.6	1.2	2.0

Note：* The ratio of diameter of transparent todiameter of agar block.

Enzymatic activities

Fifty-two representative isolates were chosen for protease and amylase activity detection. The numbers of isolates showed protease and amylase activity were 2 and 19,count for3.8% and 36.5%, respectively (Table 3). Isolate G25 could produce both protease and amylase. The ratio of the diameter of transparent toagar block can indicate the strength of enzymatic activity. Isolate G25 showed stronger protease activity than isolate G2-1. Isolate I3 showed the strongest amylaseamong all isolates (Table 3). The enzymatic activity of 7 isolates was shown in Figure 2.

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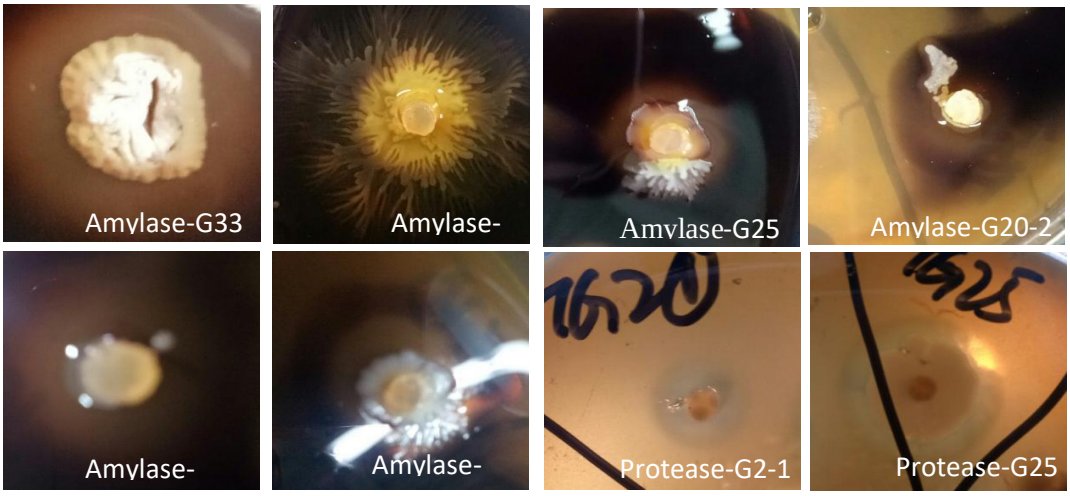

Fig 2.Enzymatic activities of several actinomycete isolates

Table 3 Enzymatic activities of actinomycete isolates

Enzymes	Isolates	Diameter of agar block (cm)	Diameter of transparent (cm)	Ratio*
Protease	G2-1	0.3	0.9	3.0
	G25	0.3	2.2	7.3
Amylase	G2-2	0.3	1.2	4.0
	G4	0.3	1.8	6.0
	G8	0.3	1.6	5.3
	G10	0.3	1.4	4.7
	G11	0.3	1.5	5.0
	G20-2	0.3	1.0	3.3
	G25	0.3	1.7	5.7
	G26-3	0.3	1.5	5.0
	G28	0.3	1.4	4.7
	G31	0.3	2.1	7.0
	G33	0.3	1.8	6.0

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T4	0.3	1.6	5.3
T6	0.3	1.5	5.0
T27	0.3	0.5	1.7
N2	0.3	2.0	6.7
N3	0.3	1.2	4.0
N4	0.3	1.0	3.3
J7	0.3	2.3	7.6
I3	0.3	2.8	9.3

Note：* The ratio of diameter of transparent todiameter of agar block.

4.DISCUSSION

Actinomycetes in plant rhizosphere soil are important sources of bioactive substances, and studies showed that a variety of actinomyces with antibacterial activity and antitumor activity have been isolated from this ecosystem(Zhanget al., 2016). *PaeconiaJishanensis* is a valuable medicinal plant resource, but no research has studied the diversity and biological activity of actinomycetes in its rhizosphere soil.Hence it is particularly necessary to carry out research on it. In order to meet the growth requirements of different actinomycetes as much as possible, 5 differentisolatingmedia were used and 82 isolates were isolated in this study. Removing the repeats from different media, 52 isolates were obtained, which were accumulated for the subsequent research of biological activity.

It is reported that actinomycetes in rhizosphere soil of medicinal plants have significant antibacterial activity (Jansoand Carter, 2010). Of the 52 tested isolates, about 21.2% showed antibacterial activity, and38.5% showedprotease and amylase activity. There were 8 isolates (G10, G20-2, g26-3, G28, G33, T4, J7 and N4) showed both antibacterial and enzymatic activities at the same time. These isolates are likely to produce some new bioactive substances. They are valuable resources for inhibiting pathogenic bacteria and fungi, as well as the extraction of protease and amylase. Further exploring of should be done.

In conclusion, there are abundant actinomycete resources in rhizosphere of *PaeconiaJishanensis*. These microbial resources should receive more attention in research and for the development for new antibiotics, antitumor and antiplant pathogen agents.

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REFERENCES

[1]Berdy J. Bioactive Microbial Metabolites: A Personal View. Journal of Antibiotics, 2005, 58:1-26.

[2] LeiZhang, HuiminXu, Baoli Zhu. Relationship between rhizospheric microorganism and plant replantation disease. Journal of microbiology, 2016, 56:1234-1241.

[3] FengxianGuo, Yue Liu, Li Tang, et al. Research status and prospect of rhizosphere microorganism of medicinal plants. Chinese journal of agricultural science and technology, 2017, 19:12-21.

[4] JingjingWang, ZhirongChen, Yingying Guan, et al. Diversity analysis of actinomycetes in deep-sea sediments in the south China sea. Bulletin of microbiology, 2016, 43:1438-1447.

[5]Hinsinger P, Marschner P. Rhizosphere—perspectives and Challenges. Plant & Soil, 2006, 283: 7-8.

[6] Schendy. Selection, isolation, identification and bioactivity of rare actinomyces in rhizosphere soil. Xiamen university, 2016.

[7] Yi Cao, Ning Lu, Xingjiang Chen, et al. Isolation, enzyme activity and functional gene detection of actinomycetes in tobacco rhizosphere. Journal of northeastern agricultural university, 2014, 45:19-23.

[8] YueWu, Xiaojun Li, Jianhong Chen, et al. Study on actinomyces diversity and antimicrobial activity in rhizosphere soil of mangrove plants in beilun estuary in Guangxi. Chinese journal of antibiotics, 2017, 42:302-310.

[9]Miyanaga S, Obata T, Onaka H, et al. Absolute Configuration and Antitumor Activity of Myxochelin A Produced by *Nonomuraeapusilla* TP-A0861. Journal of Antibiotics, 2006, 59:698-703.

[10] MingjinJi. Investigation and study of wild *PaeconiaJishanensis*. Journal of shanxi agricultural university (natural science edition), 2011, 31:418-421.

[11] FengZhang. Analysis of causes of endangered plant dwarf peony. Journal of ecology, 2003, 23: 1436-1441.

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[12] Deqing Zhou, DeqiangXu, et al. Microbiology experimental course. 3rd edition.Advanced education press, 2013.

[13]Coombs JT, Franco CM. Isolation and Identification of Actinobacteria from Surface-Sterilized Wheat Roots. Applied & Environmental Microbiology, 2003, 69:5603-5608.

[14] Min Yao. Screening of antagonistic endophyticactinomyces against tomato leaf mold and preliminary study on active substances.Northwestern university of agriculture and forestry, 2007.

[15] MeiruSi, QuanhongXue. Screening of actinomycete isolation medium and study on inhibition methods of miscellaneous bacteria. Bulletin of microbiology, 2004, 31:61-65.

[16] Yongguang Zhang, Qing Liu, Hongfei Wang, et al. Diversity and enzyme activity screening of facultative basophilic actinomyces in saline-alkali soil in Xinjiang. Journal of microbiology, 2014, 54:183-190.

[17] PanpanZhang, Sheng Qin, Bo Yuan, et al. Diversity and bioactivity of endogenous and rhizosphericactinomyces of taxuschinensis in south China. Journal of microbiology, 2016, 56:241-252.

[18]Janso J E, Carter G T. Biosynthetic potential of phylogenetically unique endophyticactinomycetes from tropical plants. Appl Environ Microbiol, 2010, 76:4377-4386.