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GFP-Based approach for analysis on soil bacteriostasis towards biocontrol agent

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ABSTRACT

Soil bacteriostasis is a widespread phenomenon which negatively affects the survival, colonization and efficiency of bacterial agents in soil. A novel green fluorescent protein (GFP)-based method was developed to assess the bacteriostasis intensity (BI) of soil towards the biocontrol agent *Pseudomonas aeruginosa* NXHG29. Transformation with GFP generated a strongly fluorescent strain, NXHG29-gfp. The population and growth of NXHG29-gfp under bacteriostatic stress were determined based on the appearance of fluorescent colonies and antibiotic resistance. By the GFP-based method, sixty samples of tobacco soil showed BIs of 8.7-29.4% against NXHG29-gfp, which positively correlated with the soil microbial diversity (*r*=0.97), pH value (*r*=0.78) and organic matter (*r*=0.78). This GFP-based method represents a convenient and accurate approach for soil bacteriostasis assay compared to standard traditional methods.

INTRODUCTION

The ability of biological control agents (BCAs) to colonize, develop and survive in the rhizosphere has been considered as a prerequisite for successful control of soil-borne phytopathogens, and the variable root colonization of BCAs was considered the main reason for their inconsistent performance (Compant et al., 2010; Maurer et al., 2013). Most BCAs could not germinate and grow normally in soils due to soil microbiostasis, which resulted inconsistent effects in their practical application (Garbeva et al., 2011; Li et al., 2011). Soil microbiostasis could be divided into fungistasis, actinostasis and bacteriostasis according to the type of the targeted microorganisms (Ho et al., 1982).

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The term "soil fungistasis" was first coined by Dobbs and Hinson to describe the widespread occurrence of inhibition of germination of fungal spores or growth of fungal hyphae in soils (Dobbs and Hinson, 1953). Fungistasis became a 'hot topic' in the 1960s and 1970s, as extensively reviewed by John Lockwood, who himself spearheaded these developments (Lockwood, 1977). Research in the 'Lockwood-era' was mainly focused on understanding the mechanism of fungistasis and the relationship with suppression of soil-borne plant diseases. In the following years, many studies were performed to address the soil microorganisms (Li et al., 2011; De Boer et al., 2003; Li et al., 2008) and their compounds (Li et al., 2007; Zou et al., 2007) involved in fungistasis. Compared to the progress in fungistasis, only a few studies were performed on actinostasis and bacteriostasis which is possibly due to lack of appropriate methods for assessment. The strength of fungistasis could be

quantified easily on slides or medium plates by counting germinated conidia or measuring growth zone in comparison to the control under microscopic observation (Lockwood, 1977).

So far, only two methods have been proposed for testing the soil bacteriostasis: the agar disc method (Davis, 1976) and the antibiotic medium method (Ho et al., 1982). In the former, the diameter difference of bacterial colony between the treatment and the control mostly used for bacteriostatic evaluation. In the latter, the difference of bacterial numbers recovered from the treatment and the control using selective medium used to determinate of the bacteriostasis.

The green fluorescent protein (GFP) gene from the jellyfish *Aequoria victoria* has proven to be a valuable molecular marker which can be expressed in many environmental organisms (Errampali et al., 1999). The mentioned gene can suggest a useful tool to develop a new method for monitoring the bacteriostasis by tagging the bacteria with the green fluorescent protein genes (*gfp*). The bacterium *Pseudomonas aeruginosa* has been widely used for controlling soil borne diseases due to its excellent root colonizing ability, catabolic versatility, and capacity to produce a wide range of active metabolites (Illakkiam et al., 2013; Kumar et al., 2009; Sathyapriya et al., 2012). In our previous study, a strain (NXHG29) of *P. aeruginosa* was isolated from cucumber rhizosphere which showed strong antifungal activity against *Fusarium oxysporum* f. sp. *cubense*, the etiologic agent of banana wilt disease (Li et al., 2012). Our preliminary experiments show that the culture broth of *P. aeruginosa* NXHG29 displayed strong nematicidal activity against second stage juveniles (J2) of *Meloidogyne incognita*. To develop a novel method for bacteriostasis analysis, the objectives of this study involved (i) tagging the bacterial strain with *gfp* gene, (ii) evaluating the bacteriostasis of tobacco soils, and (iii) analyzing the relationship between bacteriostasis and soil factors including microbial community, soil pH and organic matter.

MATERIALS AND METHODS

Construction of GFP-tagged *P. aeruginosa* NXHG29

A GFP-expressing plasmid, pSMC2, which constitutively expressed a bright mutant of GFP and stably maintained in *Pseudomonas* strains (Bloemberg et al., 1997), was kindly provided by Dr. Guido V. Bloemberg (Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusetts). Following the protocols of Choi et al. (2006), competent cells of *P. aeruginosa* NXHG29 were prepared and electroporated using the plasmid pSMC2. The transformed cells were immediately transferred into 1 mL of LB medium. After incubation at 180 rpm for 1 h at 30°C, the transformed cells were plated onto selective medium (LB agar medium supplemented with 400 µg/mL carbenicillin and 200 µg/mL ampicillin). Bacterial colonies harboring pSMC2 were identified under fluorescence microscopy (Olympus DP80) at an excitation wavelength of 488 nm.

Stability assay of *gfp*-transformed strain

One transformant (*P. aeruginosa* NXHG29-gfp) exhibiting strong fluorescence was selected for stability assay with regard to fluorescence and antagonism. The stability of *P. aeruginosa* NXHG29-gfp was evaluated fluorometrically by sub-culturing on LB medium without the antibiotics for 50 times, two days at every turn.

Antagonistic stabilities of *P. aeruginosa* NXHG29-gfp were evaluated in comparison with its parental strain as control. Briefly, bacterial strains were cultured using LB medium at 30°C for 72 h. Cell concentrations of both strains were adjusted to about 1×10^7 CFU/mL using LB broth. The phytopathogens *F. oxysporum* f. sp. *cubense* and *Phytophthora nicotianae* were obtained from Southwest Natural Germplasm Center Microbial Resources, China, and were used as test organisms for antifungal assay of *P. aeruginosa* NXHG29 using the Oxford cup method (Li et al., 2012). Nematicidal activity was assayed following the description of Huang et al. (2015) using *M. incognita* J2 as test nematode. The control treatment involved only LB medium. The test was repeated twice, with three replications for each treatment.

Soil sampling

A total of 60 soil samples were used in this study and collected from tobacco rhizosphere in Yunnan and Henan provinces of China during April to July of 2014 (Table 1). For a sample, approximately 1 kg of soil was collected randomly from the top layer (2-15 cm) over an area about 20 m^2 in a field with an area > 500 m^2. All samples were homogenized with a pulverizer, air-dried for 48 h, sieved to <2 mm, and stored in glass bottles at 4°C until used.

Soil bacteriostasis assay: GFP-based method and antibiotic medium method

P. aeruginosa NXHG29-gfp was grown on LB medium. After incubation at 30°C for 72 h, bacterial cells were harvested by centrifugation at 10,000 rpm for 10 min. The bacterial pellet was washed twice with 0.1 M phosphate buffered saline (pH 7.2) and suspended in 10% Skim Milk (Difco™). After mixed thoroughly, the suspension was frozen at -80°C for 1 h, -20°C for 1 h and -80°C for 2 h, then was lyophilized for 12 h by a freeze dryer (Eyela FDU-2100, Japan) to obtain bacterial powder. Colony

Table 1. Information of the soil samples.

Soil number	Origin	Cultivar	pH	OM (g/kg)	H´	BI (%)
KM01	Yunnan	Hongda	6.13	18.73	1.46	10.51±1.04
KM02	Yunnan	Yunyan-87	6.81	20.61	1.85	19.51±1.72
KM03	Yunnan	K-326	6.53	21.90	1.67	15.49±1.18
YX01	Yunnan	Hongda	6.47	19.64	1.51	13.93±1.54
YX02	Yunnan	Yunyan-87	6.15	15.95	1.38	11.47±1.25
YX03	Yunnan	K-326	5.91	16.07	1.42	12.35±0.95
HH01	Yunnan	Hongda	5.40	12.03	1.24	9.21±0.84
HH02	Yunnan	Yunyan-87	6.73	20.57	1.79	18.63±2.13
HH03	Yunnan	K-326	6.82	22.38	1.93	21.78±2.25
QJ01	Yunnan	Hongda	7.48	27.04	2.37	29.42±2.66
QJ02	Yunnan	Yunyan-87	6.29	21.16	1.71	17.27±1.52
QJ03	Yunnan	K326	5.81	13.07	1.26	9.73±1.06
DL01	Yunnan	Hongda	6.45	18.01	1.49	13.37±1.17
DL02	Yunnan	Yunyan 87	6.02	16.88	1.44	12.51±1.24
DL03	Yunnan	K-326	6.53	21.07	1.69	16.23±2.13
CX01	Yunnan	Hongda	6.70	23.66	2.05	28.38±2.37
CX02	Yunnan	Yunyan-87	6.94	20.94	1.58	13.27±1.54
CX03	Yunnan	K-326	6.33	18.34	1.50	13.23±1.17
NJ01	Yunnan	Hongda	6.67	17.38	1.48	12.84±1.06
NJ02	Yunnan	Yunyan 87	6.57	21.14	1.71	17.52±2.07
NJ03	Yunnan	K-326	6.42	21.51	1.73	18.13±1.87
BS01	Yunnan	Hongda	7.18	26.15	2.27	25.44±2.61
BS02	Yunnan	Yunyan-87	6.29	17.92	1.43	11.70±1.16
BS03	Yunnan	K-326	6.09	14.47	1.35	10.12±0.86
WS01	Yunnan	Hongda	6.43	17.73	1.46	12.38±1.21
WS02	Yunnan	Yunyan-87	6.24	19.48	1.59	12.43±1.53
WS03	Yunnan	K-326	6.92	22.62	1.98	22.11±2.41
ZT01	Yunnan	Hongda	6.07	14.80	1.31	9.82±0.76
ZT02	Yunnan	Yunyan-87	6.23	17.97	1.51	12.77±1.15
ZT03	Yunnan	K-326	6.85	24.56	2.26	24.94±2.52
BY01	Henan	NC-89	6.46	19.77	1.43	10.88±1.08
BY02	Henan	NC-89	6.31	18.26	1.36	10.54±0.96
BY03	Henan	Yuyan-6	6.77	20.81	1.65	13.38±1.31
BY04	Henan	Yuyan-6	6.63	18.78	1.58	12.73±1.27
BY05	Henan	Yuyan-10	6.92	20.51	1.72	18.61±1.67
BY06	Henan	Yuyan -10	6.24	18.36	1.33	9.43±0.86
BY07	Henan	Youxuan-1	6.43	17.67	1.51	11.65±1.14
BY08	Henan	Youxuan-1	6.78	21.21	1.72	17.17±1.58
BY09	Henan	Zhongyan-100	6.51	22.83	1.71	16.83±1.67
BY10	Henan	Zhongyan-100	6.70	22.68	2.11	28.44±2.64
JX01	Henan	NC-89	7.18	28.71	2.33	29.15±2.41
JX02	Henan	NC-89	6.31	17.59	1.46	10.71±1.28
JX03	Henan	Yuyan-6	6.67	18.91	1.51	11.27±1.05
JX04	Henan	Yuyan-6	5.93	14.27	1.29	8.73±0.88
JX05	Henan	Yuyan-10	6.75	19.37	2.23	27.47±2.62
JX06	Henan	Yuyan-10	6.57	19.27	1.68	18.65±1.73
JX07	Henan	Youxuan-1	7.22	27.93	2.21	26.84±2.16
JX08	Henan	Youxuan-1	6.74	18.92	2.17	25.41±2.33
JX09	Henan	Zhongyan -100	6.31	17.37	1.27	9.13±1.31

Table 1. Contd.

JX10	Henan	Zhongyan -100	6.83	20.51	1.74	19.25±1.86
XX01	Henan	NC-89	6.58	20.54	1.69	17.81±1.74
XX02	Henan	NC-89	7.22	27.65	2.37	28.62±2.84
XX03	Henan	Yuyan-6	6.44	18.97	1.37	10.27±1.12
XX04	Henan	Yuyan-6	6.76	19.42	2.07	26.12±2.51
XX05	Henan	Yuyan-10	6.37	17.90	1.35	11.18±1.44
XX06	Henan	Yuyan-10	6.54	19.81	1.71	18.87±1.37
XX07	Henan	Youxuan-1	6.83	21.37	1.92	25.73±1.62
XX08	Henan	Youxuan-1	6.77	20.88	2.11	27.31±1.29
XX09	Henan	Zhongyan-100	6.18	16.91	1.42	11.34±0.87
XX10	Henan	Zhongyan-100	6.51	18.53	1.73	20.26±1.57

Note: BI values were obtained by GFP-based method..

Table 2. The bacteriostasis intensity (BI, %) of five representative soils against *P. aeruginosa* NXHG29-gfp assayed by different methods.

Soil	GFP-based method (This study)	Antibiotic medium method (Ho and Ko, 1982)	Agar disc method (Davis, 1970)
KM01	10.51±1.04 a	7.35±0.93 b	42.48±3.21 c
DL03	16.23±2.13 a	11.24±1.25 b	53.12±2.64 c
XX10	20.26±1.57 a	14.63±1.06 b	61.88±2.71 c
XX07	25.73±1.62 a	21.35±1.58 b	68.06±3.22 c
JX01	29.15±2.41 a	22.71±2.37 b	76.51±3.57 c

The means in each lane followed by the same letter do not differ significantly according to Duncan' s multiple range test at P≤0.05.

forming units (CFU) of the bacterial powder was determined as 1.5×10^7 CFU/g by a standard dilution plate technique using the LB selective medium.

In a 50 mL glass flask, 19 g soil from each representative soil (Table 2), which showed different bacteriostasis in preliminary experiments, was mix thoroughly with 1 g bacterial powder (concentration = 1.5×10^7 CFU/g) and 5 ml LB broth. Equal amount of sterilized soil which replaced the natural soil of same sample served as control. Three replicates were prepared for each sample. After incubation of 15 d at 30°C, 1 g subsamples from treatment and control were withdrawn and CUF/g of soil of *P. aeruginosa* NXHG29-gfp determined by dilution plating method using LB selective medium. After incubation at 30°C for 48 h, the CFU emitting green fluorescence on plates was determined under fluorescence microscopy (Olympus DP71, Japan). Meanwhile, the bacterial CFU/g was also enumerated visually without fluorescence microscopy, and the result was owed to the antibiotic medium method.

The BI of soil towards *P. aeruginosa* NXHG29-gfp was calculated as: BI (%) = (CUF in control - CUF in treatment) / CUF in control×100.

Soil bacteriostasis assay: Agar disc method

Determination of bacteriostasis by agar disc method was performed following the description of Davis (1976). In a Petri dish containing 50 g of each representative soil (Table 2), 20 mL sterile water was added. The surface of the soil was smoothed and a piece of cellophane was placed upon it. *P. aeruginosa* NXHG29-gfp was grown in LB medium at 30°C for 72 h, then, 50 µL of the culture was mixed thoroughly with 100 ml of the molten, sterile, yeast peptone agar (YPA) at 40-45°C and aseptically poured into a Petri dish to a depth of 1.5 mm. Three agar discs (diameter = 8 mm) containing *P. aeruginosa* NXHG29-gfp were incubated on the cellophane of each dish. For the control, soil was replaced with LB medium. Three replications were prepared for each treatment. After incubation for 7 d at 30°C, the diameters of bacterial colonies on the discs were measured under microscope (Olympus BX51, Japan).

The BI of soil towards *P. aeruginosa* NXHG29-gfp was calculated as: BI (%) = (colony diameter of control - colony diameter of treatment) / colony diameter of control×100.

Table 3. Comparing on the antagonistic activities between *P. aeruginosa* NXHG29-gfp and *P. aeruginosa* NXHG29.

P. aeruginosa strain	Antifungal activity (%)		Nematicidal activity (%)
	F. oxysporum f. sp. *cubense*	*P. nicotianae*	
NXHG29-gfp	57.42±1.43 a	46.21±1.13 a	76.51±2.47 a
NXHG29	58.08±1.27 a	45.38±1.58 a	75.98±1.87 a

The means in each column followed by the same letter do not differ significantly according to Duncan' s multiple range test at P≤0.05.

Evaluation of microbial community-level physiological profiling

Carbon utilization was estimated using Biolog EcoPlate™ (Biolog Co., Hayward, CA) with 31 substrates in the plates and classified into six categories. For a sample, 5 g soil was shaken in 95 ml of sterile normal saline (0.85% NaCl) for 30 min at 5,000 rpm and then diluted to 1:1000. Each plate well was inoculated with 150 µl of the dilution, and the plates were incubated in the dark at 28°C for 10 days. Three replicates were prepared for each sample. Microbial growth was followed by reading the optical density (OD) at 590 nm every 24 h using a Biolog Microstation™ reader (Biolog Inc., Hayward, CA). Data was collected using the Microlog™ 4.01 software.

Average well-colour development (AWCD) was calculated as a measure of microbial functional diversity and was calculated as: AWCD =$\sum(C_i-R)/31$. Where R is the absorbance of the control well (containing water instead of C source) and C_i is the absorbance of plate well inoculated with C source i. The AWCD was assigned a value of 0 when C_i-R<0. The EcoPlate readings at 96 h were used to calculate the Shannon's diversity index (H´) using the equation:

$H' = -\sum P_i \ln(P_i)$, where $P_i = (C_i-R)/\Sigma (C_i-R)$ (Feigl et al., 2017).

Analysis of the pH and organic matter

Soil pH was measured by a pH meter (Mettler Toledo FE20, Switzerland) in water with a soil/solution ratio of 1:2.5 (w/v). Soil organic carbon (OC) content was determined according to the Walkley and Black method and the organic matter (OM) content was calculated by multiplying the OC by 1.724 (Nelson and Sommers, 1996).

Statistical analysis

All statistical analyses were performed using analysis of variance (ANOVA) with the SPSS statistical software package (Version 17.0; SPSS, Inc., Chicago, IL, USA) and Microsoft Office Excel 2010. Significant differences among treatments (P≤0.05) were determined according to Duncan's multiple range test. Data were presented as the mean ± standard deviation. In order to determine the relationships between bacteriostasis intensity and soil factors, simple linear regression analysis (y = a ± bx) was performed with the use of MS Excel and Statistica 6.0 software.

RESULTS

Stability of *P. aeruginosa* NXHG29-gfp

Under fluorescence microscopy, cells of *P. aeruginosa* NXHG29-gfp were readily visualized by their GFP fluorescence for 42 d on LB medium without the antibiotics. After sub-culturing 50 times, the intensity of GFP fluorescence remained unchanged and the growth curve of *P. aeruginosa* NXHG29-gfp was identical to the parental strain (data not shown).

The *P. aeruginosa* NXHG29-gfp suppressed the mycelial growth of *F. oxysporum* f. sp. *cubense* and *P. nicotianae* (57.42 and 46.21%), respectively, while *P. aeruginosa* NXHG29 showed the antifungal activities of 58.08 and 45.38% respectively towards the two fungal pathogens (Table 3). *P. aeruginosa* NXHG29-gfp and *P. aeruginosa* NXHG29 respectively showed the lethal rates of 76.51 and 75.98% to *M. incognita* J2 (Table 3). The *P. aeruginosa* NXHG29-gfp showed similar antifungal and nematicidal activities as its wild-type, indicating that the presence of pSMC2 did not interfere with the antagonistic activity or potential of *P. aeruginosa* NXHG29.

The comparison of soil bacteriostasis assays

The BIs of five representative soils were tested by GFP-based method, antibiotic medium method and agar disc method. The first two were used to evaluate the bacteriostasis based on the CFU change of the target strain in the soil after incubation for 15 d at 30°C. The five samples showed BIs of 10.51-29.15% when tested by GFP-based method, and 7.35-22.71% by antibiotic medium method (Table 2). For same sample, the BI values obtained by GFP-based method were significantly

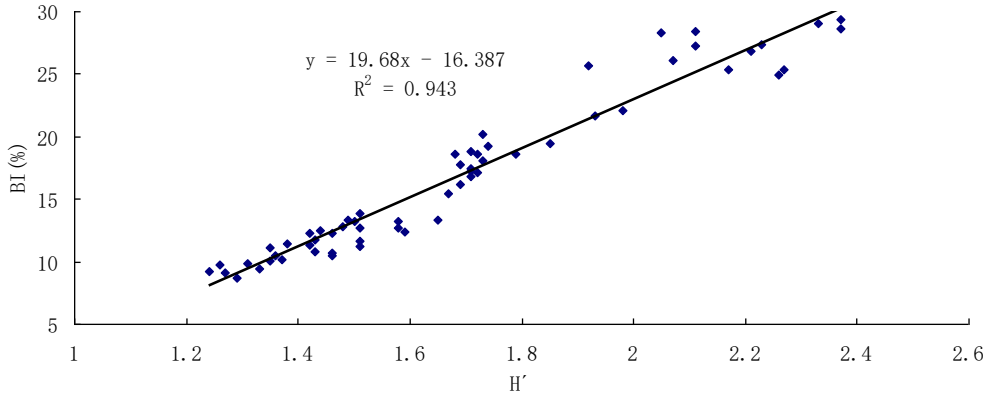

Figure 1. Analysis on the linear correlation between BI and Shannon's H´.

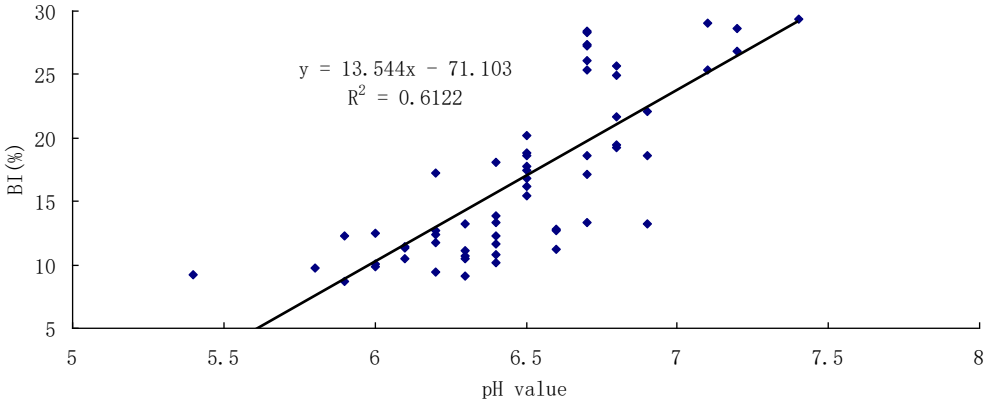

Figure 2. Analysis on the linear correlation between BI and soil pH value.

higher than that from antibiotic medium method ($p<0.05$), and the difference of BI ranged from 3.16% (KM01) to 6.44% (JX01). By agar disc method, all the five soils showed strong bacteriostasis to suppress the expansion of bacterial colonies, which generated the BIs of 42.48, 53.12, 61.88, 68.06 and 76.51%, respectively (Table 2).

Bacteriostasis of tobacco soil against *P. aeruginosa* NXHG29-gfp

The BIs of 60 tobacco soils towards *P. aeruginosa* NXHG29-gfp were examined by GFP-based method. These soils varied their BI from 8.7% (JX04) to 29.4% (QJ01) with an average of 16.8% (Table 1). For the 30 samples from Yunnan Province, China, their BI ranged from 9.2 to 29.4% (average 15.84%), while the other 30 samples from Henan Province, China showed BI of 8.7-29.1% (average 17.75%). When considering the tobacco cultivars, soils collected from Youxuan-1 showed the strangest BI (22.32%), followed by NC-89 (17.92%), Zhongyan-100 (17.5%), Yunyan-10 (17.32%), Hongda (16.49%), K-326 (16.37%), Yunyan-87 (14.67%) and Yunyan-6 (13.7%).

Correlation between bacteriostasis and soil factors

The relationships between bacteriostasis intensity and soil factors (microbial diversity, pH value and organic matter) were presented in Figures 1-3. A very strong linear correlation was observed between BI and microbial diversity, where the correlation coefficient (r) was 0.97 (Figure 1). The BI also showed strong linear correlation with soil pH value (r =0.78) (Figure 2) and soil organic matter (r =0.78) (Figure 3).

DISCUSSION

No increase in numbers of bacteria in soil in a certain period was used as an indication of the presence of bacteriostasis in soil (Brown, 1973). To assay the

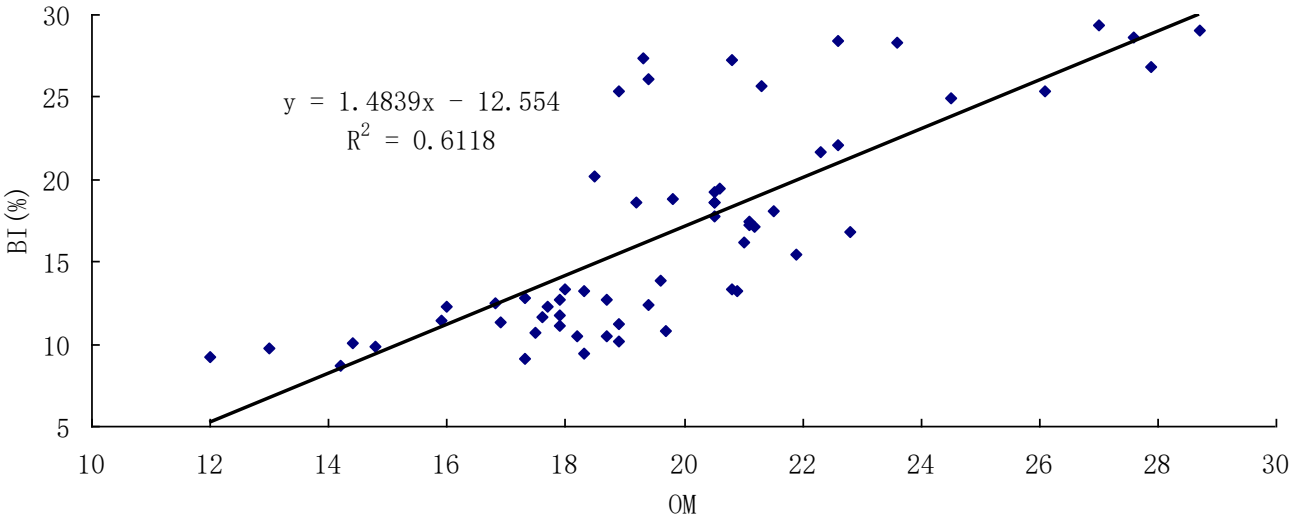

Figure 3. Analysis on the linear correlation between BI and soil OM.

bacteriostasis, Ho and Ko firstly induced the targeted bacteria *Agrobacterium radiobacter* and *Streptomyces scabies* to obtain streptomycin resistance, and then determined the bacterial propagules by agar medium supplemented with 500 µg/mL streptomycin sulphate (Ho et al., 1982). In this study, we developed a method for testing bacteriostasis by tagged *P. aeruginosa* NXHG29 with *gfp* gene. The tagged strain *P. aeruginosa* NXHG29-gfp also could be used for bacteriostasis assay based on its resistance to carbenicillin and ampicillin. However, we found that the BI values from GFP-based method were significantly higher than that from antibiotic medium method about 3.16-6.44%. By checking all the plates, we found that the CUF/g numbers counted by colony counter were higher than that determined by fluorescence microscopy. This suggested that the selective medium used (LB supplemented with 400 µg/ml carbenicillin and 200 µg/ml ampicillin) could not inhibit the propagation of all indigenous bacteria from soil. Thus, result of soil bacteriostasis obtained from GFP-based method was more accurate than that from antibiotic medium method. Additionally, it's not always possible to induce resistance for a given microorganism, which will restrict the use of the antibiotic medium method for some microorganisms. By agar disc method, Davis (Davis, 1976) tested the bacteriostasis of five soil samples towards bacteria, the BI values ranged from 68.09 to 96.89% after transforming the data of the colony diameter for BI using our formula. The BIs were higher than that generated in this study possibly due to the differences of soil, targeted bacteria and incubation conditions. The inconvenience of agar disc method was to compare the size of colony under microscope for their variation usually under 20 µm, which almost was negligible by naked eye. Additionally, the irregular colonies would be resulted in calculation error. According to our study, the GFP-based method provided us a convenient and accurate approach for bacteriostasis assay by recombinant microorganisms to be visualized, which would be valuable for the research on soil bacteriostasis in future.

In this study, 60 tobacco soils showed 8.7-29.4% of bacteriostasis to suppress population of *P. aeruginosa* NXHG29-gfp, which indicated the universality of bacteriostasis in soil. Brown (1973) reported that bacteriostasis was less active in the acidic soils. However, our results indicated that the bacteriostasis intensity was positively related to soil pH in the range of 5.4-7.48.

The inhibition (Romine and Baker, 1973) and nutrient-deficiency (Lockwood, 1977) theories have been proposed to explain the mechanism of soil fungistasis. The former states that microbial production of inhibitory compounds impedes fungal development, whereas the latter states that microbial withdrawal of nutrients limits the germination and/or growth of fungi. Both theories accept that soil microorganisms play important role in the process of fungistasis. A range of bacterial genera and their volatile compounds have been demonstrated to be involved in soil fungistasis (Li et al., 2011; De Boer et al., 2003; Li et al., 2008; Zou et al., 2007; Xu, et al., 2004). So far, no study has been performed to characterize the microorganisms that involved in soil bacteriostasis. In the present study, a strong linear correlation was observed among bacteriostasis intensity and microbial diversity (r = 0.97) and soil organic matter (r = 0.78). The input of organic matter could enhance the diversity and stability of soil microbial community (Wang, et al., 2017), which possibly increased the bacteriostasis. Yet, this only provides sketchy information regarding the relationship

between bacteriostasis and microorganisms of soil. Further researches are required to understand the taxonomy and functions of related microorganisms.

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