African Journal of Microbiology Research Vol. 5(16), pp. 2316-2324, 18 August, 2011
Available online http://www.academicjournals.org/ajmr
DOI: 10.5897/AJMR11.625
ISSN 1996-0808 ©2011 Academic Journals

Full Length Research Paper

Phylogenetic analysis of the nematicidal actinobacteria from agricultural soil of China

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Accepted 30 July, 2011

The purpose of this study was to assess the diversity and biocontrol potential of nematicidal actinobacteria from agricultural soils. Two hundred soil samples were collected from 20 provinces (autonomous regions or municipalities) of China, and 4000 actinobacteria isolates were obtained. Of the 4000 isolates evaluated, 533 (13.3% of total) and 488 (12.2%) respectively showed nematicidal properties to target nematodes *Panagrellus redivivus* and *Bursaphelenchus xylophilus* with nematicidal activities (NA) of more than 30%. The sum of strains with NAs of 90-100%, 80-90%, 60-80% and 30-60% was 55, 100, 127 and 251 to *P. redivivus*, while 37, 85, 111 and 255 to *B. xylophilus*, respectively. The most active isolates showed high host selectivity, in which only 101 isolates (5.1% of the total) were toxic to the both targets with NAs>30%. Additionally, 69 of the 101 isolates were randomly selected for species diversity analysis. Phylogenetic analysis placed the 69 actinobacteria in three families (Streptomycetaceae, Pseudonocardiaceae and Nocardiaceae) of the *Actinobacteria* with sequence similarity of 97.4-100%. The largest group was Streptomycetaceae, containing 58 isolates (84.1% of the total) that showed 97% to 100% sequence identity to 28 species of the genera *Streptomyces* (57 isolates, 27 species) and *Kitasatospora* (1, 1). The Pseudonocardiaceae group contained 7 isolates (10.1% of the total) showing 98.5-99.7% homology to *Amycolatopsis lurida* (4 isolates) and *A. niigatensis* (3). The Nocardiaceae group included 4 isolates (5.8% of the total) with 99-99.6% sequence identity to one species *N. fluminea*.

Key words: Nematicidal actinobacteria, nematode, biocontrol, phylogenetic analysis.

INTRODUCTION

Plant parasitic nematodes cause damages to a variety of agricultural crops throughout the world. Only the root-knot nematodes (*Meloidogyne* spp.) cause about US$100 billion loss annually to a wide variety of crops worldwide (Oka et al., 2009). Currently, the application of chemical nematicides and fumigants is still the main strategy to control these pathogens. Although chemical nematicides are effective, easy to apply, and show rapid effects, they have begun to be withdrawn from the market in some developed countries owing to concerns about public health and environmental safety problems (Schneider et al., 2003). At present, several successful biocontrol agents mainly using nematophagous fungi have been used widely for nematode diseases (Tikhonov et al., 2002). As antagonists of parasitic nematodes, nematophagous fungi exhibited their biocontrol traits by

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predation, parasitism or poisonous effects.

Extracellular enzymes of nematophagous fungi, especially the serine protease, served as the vital virulence factor in the process of infection (Huang et al., 2004). A few species of antagonistic bacteria have been developed into agents against plant parasitic nematodes, e.g. *Bacillus thuringiensis* and *Pasteuria penetrans* (Chen and Dickson, 1998; Javed et al., 2008). *B. thuringiensis* exhibited toxic effects against invertebrate by its parasporal inclusion and has been used widely (Wei et al., 2003; Meadows et al., 1989). *P. penetrans* is an obligate parasite of root knot nematodes (*Meloidogyne* spp.), but its fastidious life cycle and the obligate nature of parasitism have inhibited its mass culturing and deployment in field conditions. Actinobacteria, however, have so far received relatively little attention as potential biocontrol agents against plant-parasitic nematodes.

Actinobacteria is a group of gram positive bacteria with a G+C content of over 55%. More than 70% of bioactive compounds are produced from these microorganisms and they have shown significant applications in pharmacy, industry, agriculture and environmental protection. Members of this group are best known for their ability to produce lytic enzymes, various secondary metabolites, including antibiotics. For example, avermectins includes a series of macrocyclic lactone derivatives produced by *Streptomyces avermitilis* that belongs to a new family of potent antihelminthic agents (Burg et al., 1979). Since the early 1980s, the avermectins and their derivatives have been used widely in the world to control parasitic nematodes and pests. The discovery and the huge commercial market of avermectins promoted an increasing number of researchers to study the biocontrol potential of actinobacteria. For example, Mishra et al. (1987) found that metabolites from 15 isolates of aerobic actinobacteria (screened from 502 actinobacteria) were toxic to the free-living nematode *Panagrellus redivivus* (Mishra et al., 1987).

A species of the genus *Streptomyces* isolated from nematode suppressive soil inhibited the reproduction of *Caenorhabditis elegans* in the laboratory test and reduced tomato root galling caused by *M. incognita* in the greenhouse experiment (Dicklow et al., 1993). Some nematicidal actinobacteria were observed in eggs and cysts of *Heterodera glycines* (Nour et al., 2003) and *H. trifolii* (Hay and Skipp, 1993). Sun et al. (2006) isolated 30 actinobacteria from root-knot samples and found that 47% of them were virulent to eggs and juveniles of *M. hapla*.

These investigations indicated that nematicidal actinobacteria are abundant in agricultural environments. However, no study has been performed to analyze the diversity and phylogenetic relationships of nematicidal actinobacteria from a wide range of agricultural soils. The objective of this study was to estimate the relative biocontrol potential of actinobacteria isolated from 20 provinces (municipalities or autonomous regions) of China. We also presented a phylogenetic analysis to characterize these nematode-antagonistic microorganisms.

MATERIALS AND METHODS

Soil sampling

A total of 200 agricultural soil samples were collected from 20 provinces (municipality or autonomous regions) of China (Figure 1). In each province (municipality or autonomous region), 10 samples were collected. For each sample, approximately 1 kg of soil was sampled randomly from the top layer (2-15 cm) over an area of more than 5 m^2 per field. Soil samples were spread out to air dry at room temperature for 2-3 days and sieved through a 2 mm sieve, then stored in glass bottles at 4°C until used.

Isolation of actinobacteria

To isolate the soil actinobacteria, 1 g of each dry sample was suspended in 10 m1 sterile distilled water and diluted 1000-fold. 0. l ml of the dilutions was spread on ISP5 medium (ISP5 medium: L-asparagine 1g, glycerol 10g, K_2HPO_4 1 g, trace salts 1 ml, agar 18 g, pH 7.2, added to 1000 ml with ddH_2O, where the trace salts including $FeSO_4 \cdot 7H_2O$ 0.2 g, $MnCl_2 \cdot 2H_2O$ 0.1 g, $ZnSO_4 \cdot 7H_2O$ 0.1 g, added to 100 ml with ddH_2O). The plates were incubated at 28°C for 2 weeks and actinobacteria were purified from single colonies using the same medium.

Nematode inocula

In this study, a free-living nematode, *Panagrellus redivivus*, and a pinewood nematode, *Bursaphelenchus xylophilus*, were used as target hosts for assaying the *in vitro* nematicidal activity of the actinobacteria. *P. redivivus* was cultured on autoclaved oatmeal as previously described (Walker and Barrett, 1991) and *B. xylophilus* was fed with a fungus as described by Dong et al. (2004). The juveniles were separated from media through the Baermann funnel technique (Gray, 1984), and suspended in the water for use.

Nematicidal activity assay of actinobacteria

Each purified isolate was inoculated into 50 ml of ISP5 liquid medium in a 150 ml flask. After incubation of 2 weeks at 28°C, 200 rpm, the culture suspension without actinobacteria was obtained by sterile filtration and used for nematicidal activity assay. Briefly, 1 ml of the culture suspension was added into a well of a 24-well cell culture plate, and 0.1 ml nematode suspension (approximate 150 juveniles) was added and mixed. After incubation at 28°C for 24 h, the mobile (live) and immobile juveniles were recorded by counting >150 individuals under a microscope. Those immobile juveniles were taken as dead when they could not revive within 12 h after being transferred to fresh WA and subsequently to tap water. Each treatment was replicated three times, and the experiment was run three times.

Sequence generation and phylogenetic analysis

Genomic DNA of actinobacteria was extracted using a bacterial genomic DNA extraction kit (BioTeke Corporation, China, Cat#:DP2001) and their 16S rRNA genes were amplified by PCR using the universal primer combination 27f (5′ AGA GTT TGA TCC TGG CTC AG 3′) and 1492r (5′ GGT TAC CTT GTT ACG ACT T 3′). The amplified products were purified using the Agarose gel DNA

Figure 1. Location of the 20 sampling sites (∗) in this study distributed on the map of China, which including two municipalities (BJ and SH), six autonomous regions (GX, NM, NX, XJ and XZ), and twelve provinces (Abbreviation meanings on the map, BJ: Beijing, GS: Gansu, GD: Guangdong, GX: Guangxi, GZ: Guizhou, HN: Hainan, HeB: Hebai, HeN: Henan, HLJ: Heilongjiang, HuN: Hunan, JL: Jilin, JS: Jiangsu, LN: Liaoning, NM: Neimenggu, NX: Ningxia, SD: Shandong, SX: Shanxi, SH: Shanghai, SC: Sichuan, XZ: Xizang).

purification kit (TakaRa, code DV805A) and submitted to Beijing Genomics Institute for sequencing. The resulting sequences of 16S rRNA gene were compared with those available in the GenBank using the BLAST network service to determine their phylogenetic affiliation. Multiple alignments and sequence evolutionary distance calculations were carried out using CLUSTAL X version 2.0 (Thompson et al., 1997). Phylogenetic analysis was performed using the MEGA software packages (Kumar et al., 2004), with gaps treated as missing data. Clustering was performed using the Neighbour-Joining method (Saitou and Nei, 1987). Bootstrap analysis was used to evaluate the tree topology of the Neighbour-Joining data by performing 1,000 resamplings (Felsenstein, 1985). The 16S rRNA gene sequences have been deposited in the GenBank database under accession numbers GQ357927-GQ357995.

Data analysis

Nematicidal activity (NA) was calculated using the formula: NA = IN/SN×100%, where IN represents the number of immobile nematodes and SN represents the sum of all nematodes counted (SN > 100). Data were analyzed using the analysis of variance (ANOVA), and means were compared by the test of least significant difference (LSD) at P=0.05 using SPSS 11.0 for Windows (SPSS Inc., Chicago, USA).

RESULTS

Nematicidal activity of actinobacteria

From each soil sample, 20 strains of actinobacteria were selected for nematicidal bioassay and these strains were selected mainly based on the differences of colony features, or randomly picked if colony features were similar, which resulting in a total of 4000 strains from 200 samples for virulence test. Of the 4000 isolates tested, 533 (13.2% of total) exhibited nematicidal activities with NA values more than 30% against the free-living nematode *P. redivivus*. These isolates showed

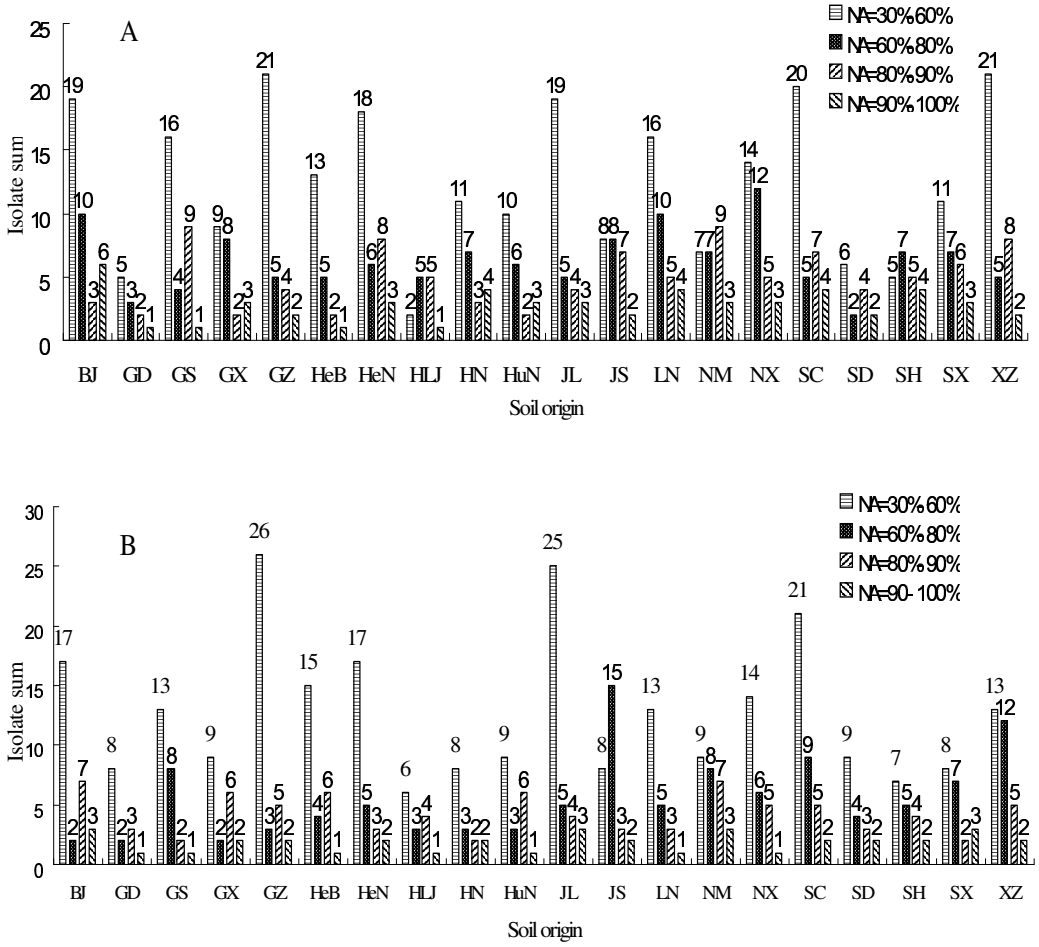

Figure 2. Statistics of nematicidal actinobacteria from different sampling sites with different nematicidal activity (NA) to nematodes *P. redivivus* (A) and *B. xylophilus* (B).

significant differences on their NAs against *P. redivivus,* which consisted of 55 isolates with NAs of 90-100%, 100 with NAs of 80-90%, 127 with NAs of 60-80%, and 251 with NAs less than 60% but more than 30% (Figure 2).

Bioassay of the 4000 actinobacteria to plant-parasitic nematode *B. xylophilus* also was performed and 488 isolates (12.2% of total) showed nematicidal virulence with NAs of 30-100%. The numbers of active isolates with NAs of 90-100%, 80-90%, 60-80% and 30-60% were 37, 85, 111 and 255, respectively (Figure 2).

Among the 4000 actinobacteria evaluated, the most active isolates showed the highest host selectivity to *P. redivivus* or *B. xylophilus*. Only 101 isolates (2.5% of total) were toxic to both targets with NA>30%. Among the 101 strains, 4 (SDYM18, SDYM7, NMYC10 and NMCG3) showed the most potency to produce nematicidal metabolites with high NAs of more than 80% against both nematodes. Of the 155 actinobacteria strains with NAs > 80% to *P. redivivus*, only 18 displayed similar NAs to both targets without statistically significant differences (P >0.05), while 54 isolates had no nematicidal activity against *B. xylophilus*. Similarly, of the 125 strains with NAs>80% to *B. xylophilus*, the NAs of 11 isolates to the two tested nematodes were not significantly different (P>0.05) while 41 isolates had no nematicidal activity against *P. redivivus*.

The four potential strains (SDYM18, SDYM7, NMYC10 and NMCG3) causing high mortality against both hosts were assayed further after diluting the culture suspension by 2, 5 and 10 folds. Our results indicated that the original culture broths of the four actinobacteria exhibited powerful virulence (85.2%≤NA≤100%) to both *P. redivivus* and *B. xylophilus*, but the former was more susceptible than the latter (P<0.05) (Table 1). There was a negative correlation between nematicidal activity and dilution factor (P<0.05) (Table 1).

Phylogenetic characterization of nematicidal actinobacteria from agricultural soil

Of the 101 isolates that showed nematicidal activities to both targets, 69 were randomly selected for phylogenetic analysis. Their 16S rRNA genes were successfully

Table 1. Nematicidal activity (NA) of four potential actinobacteria.

Strain	Nematode	Mean NA (%)±SD under different diluent			
		Origin broth	2 fold	5 fold	10 fold
SDYM18	*P. redivius*	100±0 [a]	93.2±1.22	80.8±0.83	64.2±0.85
	B. xylophilus	95.7±0.72 [b]	89.3±0.52	78.2±1.04	50.6±0.91
SDYM7	*P. redivius*	99.4±1.31 [a]	72.6±0.87	57.7±0.88	44.3±1.21
	B. xylophilus	88.3±0.89 [c]	71.7±1.11	51.6±0.62	37.2±0.82
NMYC10	*P. redivius*	99.2±1.11 [a]	80.5±0.85	68.7±0.93	34.3±1.12
	B. xylophilus	83.2±1.14 [d]	79.8±1.21	50.8±1.08	34.8±0.94
NMCG3	*P. redivius*	99.6±1.42 [a]	80.3±1.13	67.5±0.89	50.3±1.34
	B. xylophilus	85.2±0.26 [c]	75.6±0.92	56.8±1.22	41.3±0.82

Means in the "Origin broth" column followed by the same letter do not differ significantly at $p \leq 0.05$, according to multiple comparisons.

amplified using primer pair of 27f and 1492r, and submitted for sequencing. The resulting sequences, about 1380 nucleotides, were compared with those available in GenBank using the BLAST network service (Table 2), and a phylogenetic tree was constructed to determine their approximate taxonomic affiliation (Figure 3). Phylogenetic analysis placed the 69 strains in three families (*Streptomycetaceae*, *Pseudonocardiaceae* and *Nocardiaceae*) of the *Actinobacteria* with sequence similarity of 97-100% (Figure 3 and Table 2).

The largest group was *Streptomycetaceae* and it consisted of 58 isolates (84.1% of the total) showing 97 to 100% sequence identity to 27 species of the genera *Streptomyces* and 1 species of *Kitasatospora*. Among the *Streptomyces* strains, the isolates phylogenetically related to species of *S. althioticus* (8 isolates), *S. flavotricini* (7) and *S. diastatochromogenes* (6) dominated, with a total of 21 isolates (36.8% of the *Streptomyces* group). There were 13 isolates (accounting to 22.8% of *Streptomyces* members) respectively affiliated to *S. xanthophaeus* (4 isolates), *S. lateritius* (3), *S. exfoliatus* (3) and *S. ciscaucasicus* (3). Six isolates respectively identified as *S. showdoensi* (2 isolates), *S. corchorusii* (2) and *S. viridochromogenes* (2). The remaining 17 isolates were respectively identified belonging to 17 different species of the genus *Streptomyces*.

The *Pseudonocardiaceae* group contained 7 isolates (10.1% of the total) respectively showing 98.5-99.7% sequence identity to species of *Amycolatopsis lurida* (4 isolates) and *A. niigatensis* (3). The last group, *Nocardiaceae*, only included 4 isolates (5.8% of the total) with 99-99.6% identity to a species *N. fluminea*.

Of the four isolates showed strong nematicidal activity, NMYC10 and NMCG3 respectively showed 98.9 and 99.3% sequence identity to *S. diastatochromogenes* and *S. violaceochromogenes*, while SDYM18 and SDYM7 were assigned to the same species *A. lurida* with the homology more than 99%.

DISCUSSION

In this study, 4000 actinobacteria isolated from agricultural soils of a wide range of geographical regions were evaluated for their nematocidial activities. The proportions of strains capable of killing *P. redivivus* and *B. xylophilus* were 13.3 and 12.2% respectively when taking NAs≥30% as a cutoff for virulence activity. These results indicated that there were a large number of nematocidial actinobacteria distributed in agricultural soils. Of the 69 active actinobacteria submitted for phylogenetic analysis, 57 (accounted to 82.6%) showed 97 to 100% homology to 27 species of the genus *Streptomyces*. Ruanpanun et al. (2010) isolated 83 actinobacteria from plant-parasitic nematode infested soils and found the predominant actinobacteria taxa was *Streptomyces* (97.6%). Similarly, Luo et al. (2006) reported that in the 20 actinobacteria isolated from eggs and females of root-knot nematodes, 16 strains were members of *Streptomyces*. Most actinobacteria commonly isolated from soil belonged to the genus *Streptomyces* because they are more common in the environment and tend to have rapid growth rate and good sporulation compared to other actinobacteria (Williams and Vickers, 1988). This may explain why the nematicidal *Streptomyces* spp. had high occurrence frequencies. Beside the biocontrol application of *S. avermitilis* (Jayakumar, 2009; Wright et al., 1983), the producer of avermectins, many other species of *Streptomyces* also had the potential as biocontrol agents against parasitic nematodes. CR-43^T, a strain of *S. costaricanus* isolated from a nematode-suppressive soil, exhibited both antinematodal and antifungal activities in laboratory, greenhouse, and field trials (Dicklow et al., 1993). An application for a patent for CR-43^T as a

Table 2. Nematicidal actinobacteria used for phylogenetic analysis in this study and their closest affiliation according to the partical 16S rRNA gene.

Strain [Accession No.]	Sample location	The closest relative in the database [Accession No.]	Similarity (%)
SDYM9 [GQ357978]	Shandong	*S. lateritius* LMG 19372^T [AJ781326]	99.8
BJH10 [GQ357963]	Beijing	*S. lateritius* LMG 19372^T [AJ781326]	99.9
SCTS20 [GQ357973]	Sichuan	*S. lateritius* LMG 19372^T [AJ781326]	99.9
HNHS7 [GQ357938]	Henan	*S. exfoliatus* NBRC 13191^T [AB184324]	99.8
SXYC14 [GQ357967]	Shanxi	*S. exfoliatus* NBRC 13191^T [AB184324]	99.6
HNYM7 [GQ357937]	Henan	*S. exfoliatus* NBRC 13191^T [AB184324]	99.9
NXFQ8 [GQ357970]	Ningxia	*S. griseoplanus* AS 4.1868^T [AY999894]	99.9
NMXM7 [GQ357930]	Neimenggu	*S. kurssanovii* NBRC 13192^T [AB184325]	99.9
BJS2 [GQ357965]	Beijing	*S. mauvecolor* LMG 20100^T [AJ781358]	99.1
GSPG8 [GQ357950]	Gansu	*S. xanthophaeus* NBRC 12829^T [AB184177]	99.7
SCYT16 [GQ357972]	Sichuan	*S. xanthophaeus* NBRC 12829^T [AB184177]	99.7
SCLZ7 [GQ357981]	Sichuan	*S. xanthophaeus* NBRC 12829^T [AB184177]	99.8
SCL7 [GQ357982]	Sichuan	*S. xanthophaeus* NBRC 12829^T [AB184177]	99.9
NXHG4 [GQ357980]	Ningxia	*S. flavotricini* NBRC 12770^T [AB184132]	99.9
GSPT14 [GQ357949]	Gansu	*S. flavotricini* NBRC 12770^T [AB184132]	99.9
HNDD1 [GQ357943]	Henan	*S. flavotricini* NBRC 12770^T [AB184132]	99.9
NXHG3 [GQ357983]	Ningxia	*S. flavotricini* NBRC 12770^T [AB184132]	99.8
HNGL19 [GQ357939]	Henan	*S. flavotricini* NBRC 12770^T [AB184132]	99.3
NXLJ11 [GQ357979]	Ningxia	*S. flavotricini* NBRC 12770^T [AB184132]	99.9
NXFQ2 [GQ357928]	Ningxia	*S. flavotricini* NBRC 12770^T [AB184132]	99.9
GSSZ10 [GQ357948]	Gansu	*S. showdoensis* NBRC 13417^T [AB184389]	98.2
GSSZ14 [GQ357953]	Gansu	*S. showdoensis* NBRC 13417^T [AB184389]	98.2
BJL6 [GQ357961]	Beijing	*S. ciscaucasicus* NBRC 12872^T [AB184208]	99.8
GZ22 [GQ357951]	Guizhou	*S. ciscaucasicus* NBRC 12872^T [AB184208]	99.7
XZYM3 [GQ357971]	Xizang	*S. ciscaucasicus* NBRC 12872^T [AB184208]	99.9
SHJ24 [GQ357969]	Shanghai	*S. longwoodensis* LMG 20096^T [AJ781356]	99.3
HaNXJ7 [GQ357947]	Hainan	*S. corchorusii* NBRC 13032^T [AB184267]	97.9
HaNYM16 [GQ357944]	Hainan	*S. corchorusii* NBRC 13032^T [AB184267]	97.4
CZYS9 [GQ357960]	Xizang	*S. mirabilis* NBRC 13450^T [AB184412]	100
SHDQ4 [GQ357977]	Shanghai	*S. cinereoruber* subsp. *fructofermentans* NBRC 15396^T [AB184647]	98.8
BJH1 [GQ357966]	Beijing	*S. phaeochromogenes* NBRC 3180^T [AB184738]	99.9
SHLJ2 [GQ357976]	Shanghai	*S. diastatochromogenes* ATCC 12309^T [D63867]	98.6
BJS8 [GQ357964]	Beijing	*S. diastatochromogenes* ATCC 12309^T [D63867]	99.4
SHLJ5 [GQ357975]	Shanghai	*S. diastatochromogenes* ATCC 12309^T [D63867]	99.1
GXLY20 [GQ357952]	Guangxi	*S. diastatochromogenes* ATCC 12309^T [D63867]	98.7
NMYC10 [GQ357929]	Neimenggu	*S. diastatochromogenes* ATCC 12309^T [D63867]	98.9
SXTD7 [GQ357968]	Shanxi	*S. diastatochromogenes* ATCC 12309^T [D63867]	99.3
NMCG3 [GQ357931]	Neimenggu	*S. violaceochromogenes* NBRC 13100^T [AB184312]	99.3
JLLJ17 [GQ357927]	Jilin	*S. flavoviridis* NBRC 12772^T [AB184842]	99.9
DLHG2 [GQ357958]	Liaoning	*S. viridodiastaticus* NBRC 13106^T [AB184317]	99.8
DLDG2 [GQ357959]	Liaoning	*S. longispororuber* NBRC 13488^T [AB184440]	99.3
GSL5 [GQ357956]	Gansu	*S. coeruleofuscus NBRC 12757T [AB184840]*	99.6
BJL2 [GQ357962]	Gansu	*S. alboflavus NBRC 3438T [AB184775]*	99.9
HaHD12 [GQ357942]	Hainan	*S. coelicoflavus NBRC 15399T [AB184650]*	99.9
DLTS7 [GQ357957]	Liaoning	*S. viridochromogenes NBRC 3113T [AB184728]*	99.6
JLIM5 [GQ357940]	Jilin	*S. viridochromogenes NBRC 3113T [AB184728]*	99.5
HaNYM8 [GQ357945]	Hainan	*S. malaysiensis ATB-11T [AF117304]*	99.9

Table 2. Contd.

HaNXJ11 [GQ357946]	Hainan	*S. glauciniger* NBRC 100913^T [AB249964]	99.4
JLGC14 [GQ357941]	Jilin	*S. variabilis* NBRC 12825^T [AB184884]	99.9
JLYM14 [GQ357933]	Jilin	*S. althioticus* NRRL B-3981^T [AY999791]	98.3
JLFQ8 [GQ357936]	Jilin	*S. althioticus* NRRL B-3981^T [AY999791]	97.5
GSPG5 [GQ357954]	Gansu	*S. althioticus* NRRL B-3981^T [AY999791]	97.6
JLYM15 [GQ357932]	Jilin	*S. althioticus* NRRL B-3981^T [AY999791]	97.8
JLFQ12 [GQ357935]	Jilin	*S. althioticus* NRRL B-3981^T [AY999791]	97.6
GSPG4 [GQ357955]	Gansu	*S. althioticus* NRRL B-3981^T [AY999791]	97.6
JLYM2 [GQ357934]	Jilin	*S. althioticus* NRRL B-3981^T [AY999791]	97.8
SHN26 [GQ357974]	Shanghai	*S. althioticus* NRRL B-3981^T [AY999791]	97.4
BJY6 [GQ357985]	Beijing	*N. fluminea* S1^T [AF277204]	99
SXYM10 [GQ357986]	Shanxi	*N. fluminea* S1^T [AF277204]	99.4
SXS5 [GQ357987]	Shanxi	*N. fluminea* S1^T [AF277204]	99.6
NMBC12 [GQ357988]	Neimenggu	*N. fluminea* S1^T [AF277204]	99.3
DLYT2 [GQ357984]	Liaoning	*K. arboriphila* HKI 0189^T [AY442267]	99.7
HaNHS4 [GQ357989]	Hainan	*A. niigatensis* LC11^T [AB248537]	98.5
HaNHS8 [GQ357992]	Hainan	*A. niigatensis* LC11^T [AB248537]	98.5
HaNHS12 [GQ357991]	Hainan	*A. niigatensis* LC11^T [AB248537]	99.1
NXQZ2 [GQ357995]	Ningxia	*A. lurida* DSM 43134^T [AJ577997]	99.3
SDYM18 [GQ357994]	Shandong	*A. lurida* DSM 43134^T [AJ577997]	99.4
GSPG7 [GQ357993]	Gansu	*A. lurida* DSM 43134^T [AJ577997]	99.7
SDYM7 [GQ357990]	Shandong	*A. lurida* DSM 43134^T [AJ577997]	99.7

A: Amycolatopsis, K: Kitasatospora, N: Nocardia, S: Streptomyces.

biocontrol agent for nematodes was filed by Research Corporation Technologies, Tucson, Ark, under exclusive license from the University of Massachusetts at Amherst (Esnard et al., 1995). Several strains of *Streptomyces* with antifungal activities also significantly reduced root-lesion nematode population densities in roots of both susceptible and resistant alfalfa varieties grown in field (Samac and Kinkel, 2004). Several species of *Streptomyces*, e.g. *S. dicklowii* (US Patent 5549889), *S. cyaneogriseus* (US Patent 5030650), have been patented as nematicidal biopesticides (http://www.patentstorm.us).

Many other actinomycete genera also produced biologically active secondary metabolites of medical importance and showed promising biological activity including parasitism and antibiosis (Goodfellow and O'Donnell, 1989). Rickards et al. (1998) reported that a strain belonged to the genera *Amycolatopsis* or *Amycolata* could produce the cyclic decapeptide antibiotic quinaldopeptin with nematicidal activity. A strain of *Streptoverticillium albireticuli* was found to show strong nematicidal activity against *Caenorhabditis elegans* and several fungal pathogens. This species exhibited hyphal growth on both external and internal surfaces of *C. elegans* and ultimately killed the nematode (Park et al., 2002). Of the 4 isolates with strong nematicidal activity in this study, NMYC10 and NMCG3 showed 98.9 and 99.3% homology to *S. diastatochromogenes* and *S. violaceochromogenes* respectively, while the other two (SDYM18 and SDYM7) were assigned to *A. lurida*. To isolate a wider spectrum of actinobacteria, pretreating soils such as dry-heating (Nonomura and Ohara, 1969), using *Streptomyces*' specific lytic actinophage (Kurtböke et al., 1992), and applying selective media supplemented with antibiotics (Williams et al., 1993) could all be employed.

In this study, we used a free-living nematode and a plant parasitic nematode as target for bioassay. Among the 533 isolates and 488 isolates respectively showed nematicidal activity to *P. redivivus* and *B. xylophilus*, only 101 isolates showed nematicidal properties to both target hosts. The host selectivity or sensitivity between actinobacteria and nematodes suggested that the nematicidal pathways or microbial actions were diverse. To determine the control targets of a nematicidal biopesticide, the host specificity should be taken into account. Additionally, the nematicidal actinobacteria in this study were screened based on the bioassay using culture broth. The metabolite composition of the four potential strains and their control effects in fields warrant further studies.

ACKNOWLEDGEMENTS

This work was funded jointly by "National Key Sciences and Technology Program for Water Solutions" (2009ZX07102-004-04-01; 2012ZX07102-003), NSFC

Figure 3. Phylogenetic dendrogram obtained by neighbour-joining method of 16S rRNA gene sequences. Numbers at nodes are bootstrap values based on 1000 resamplings. Bar, 1% sequence divergence. *Bifidobacterium psychraerophilum* T16^T (AY174108) and *Bifidobacterium mongoliense* DSM 21395^T (AB433856) are used as outgroups. GenBank Accession Numbers for each strain are indicated in parentheses adjacent to each strain name.

(30970100), ZhengZhou Tobacco Research Institute (122009CZ0420), Yunnan Corporation of CNTC (2011YN03), National Development and Reform Commission, and Department of Science and Technology of Yunnan Province.

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