Systematic Identification of Pathogenic *Streptomyces* sp. AMCC400023 That Causes Common Scab and Genomic Analysis of Its Pathogenicity Island

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ABSTRACT

Potato scab, a serious soilborne disease caused by *Streptomyces* spp., occurs in potato-growing areas worldwide and results in severe economic losses. In this paper, the pathogenicity of *Streptomyces* strain AMCC400023, isolated from potato scabs in Hebei Province, China, was verified systematically by the radish seedling test, the potato tuber slice assay, the potted back experiment, and the detection of phytotoxin thaxtomin A. Morphological, physiological, and biochemical characteristics were determined, and the 16S ribosomal RNA analyses of *Streptomyces* sp. AMCC400023 were carried out. To obtain the accurate taxonomic status of the pathogen strain, the whole genome was sequenced, and the phylogenetic tree among 31 *Streptomyces* genomes was formed. The average nucleotide identity (ANI) and in silico DNA-DNA hybridization (isDDH) were analyzed, and at the same time, the toxicity-related genes between *Streptomyces* sp. AMCC400023 and *Streptomyces scabiei* were compared, all based on the whole-genome

level. All of the data supported that, instead of a member of *S. scabiei*, test strain *Streptomyces* sp. AMCC400023 was a distinct phytopathogen of potato common scab, which had a relatively close relationship with *S. scabiei* while separating clearly from *S. scabiei* at least in the species level of taxonomic status. The complete pathogenicity island (PAI) composition of *Streptomyces* sp. AMCC400023 was identified, which contained a toxin region and a colonization region. It was conjectured that the PAI of *Streptomyces* sp. AMCC400023 might be directly or indirectly acquired from *S. scabiei* 87-22 by horizontal gene transfer, or at the very least, there was a very close homologous relationship between the two pathogens as indicated by a series of analyses, such as phylogenetic relationships among 31 *Streptomyces* species, ANI and isDDH analyses, PAI structure mapping, thaxtomin A synthetic gene cluster tree construction, and most important, the collinearity analysis at the genome level.

Members of the genus *Streptomyces* are gram-positive, filamentous actinomycetes that are distributed widely in nature, especially in soil, and they have genomes with very high G + C content (Chapleau et al. 2016; Zhang et al. 2016a). *Streptomyces* species are capable of producing biologically active secondary metabolites, including multiple widely used antibiotics (Bentley et al. 2002; Bérty 2005), and catabolic enzymes, enabling these bacteria to adapt well to a variety of complex soil environments (Bertram et al. 2004; Hodgson 2000). A small number of *Streptomyces* spp. are pathogens that can cause plant diseases (Loria et al. 2006). Potato scab is a global disease caused by *Streptomyces* species, causing pitted, raised, or superficial lesions on the surfaces of tuber crops, such as potatoes, beets, radishes, carrots, and peanuts, resulting in severe economic losses (Loria et al. 2006). *S. scabiei* was the first pathogen to be described in 1891, and it is distributed worldwide (Lambert and Loria 1989b). In the more than 100 years since, several pathogenic *Streptomyces* species have been reported. Among these species, *Streptomyces turgidiscabies* (Miyajima et al. 1998) and *S. acidiscabies* (Lambert and Loria 1989a) have been reported as separate pathogens in Japan and the United States. Currently, the best characterized scab-causing pathogens are

S. scabiei, *S. turgidiscabies*, *S. acidiscabies*, and *Streptomyces ipomoeae* (Loria et al. 2008). In addition to the four main pathogenic bacteria mentioned above, there are other reported pathogens that cause common scab, namely *S. europaeiscabiei*, *S. stelliscabiei*, *S. luridiscabiei*, *S. puniscabiei*, *S. niveiscabiei*, *S. reticuliscabiei*, *S. caviscabiei*, and *S. bottropensis* (Bouchek-Mechiche et al. 2000; Goyer et al. 1996; Park et al. 2003; Zhou et al. 2016).

The major causative agent of potato scab is a family of toxins known as thaxtomins (Loria et al. 2006). Thaxtomins are substances with a 4-nitroindol-3-yl-2,5-dioxopiperazine scaffold (Lerat et al. 2009). A total of 11 thaxtomin family members have been isolated. These thaxtomins are distinguished by the presence or absence of *N*-methyl or hydroxyl groups (Bignell et al. 2010a). Among these compounds, thaxtomin A (ThxA), a nitrated 2,5-diketopiperazine, is the main toxin form produced by *S. scabiei*, *S. acidiscabies*, and *S. turgidiscabies* (Healy et al. 2000; King et al. 1989; Lawrence et al. 1990), whereas the toxin produced by the pathogen *S. ipomoeae* is thaxtomin C, a nonhydroxylated homolog of ThxA (King and Lawrence 1994). Thaxtomins induce a series of changes in plant host phenotypes, which can reduce the crystalline cellulose content in the cell walls of plants; affect the expression of genes associated with cell wall synthesis; cause cell hypertrophy, root growth retardation, and Ca²⁺ and H⁺ influx in plants; and result in tissue necrosis and programmed cell death (Bignell et al. 2010a; Johnson et al. 2007; Loria et al. 2008). The *necl* gene encodes a secreted protein that is a newly identified type of toxic protein. This protein is necessary for the colonization of plant hosts by pathogenic bacteria. The main function of this protein might be to inhibit the defensive response of plants during pathogenic infection (Bignell et al. 2010a). The *tomA* gene encodes a homolog of tomatinase (Bignell et al. 2010a), which is mainly found in

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phytopathogenic fungi, and the target of this protein is an antibiotic alkaloid that participates in detoxification during pathogenic infection of the host (Braun et al. 2017). Nec1 and TomA are not required for pathogenicity, but these proteins may play an important role during the infection process (Joshi et al. 2007a; Seipke and Loria 2008). *S. turgidiscabies* also contains the *fas* operon, which is adjacent to the thaxtomin synthesis cluster and homologous to the *fas* operon in *Rhodococcus fascians* (Joshi and Loria 2007). Fas proteins mediate the synthesis and modification of cytokinins, which may be closely associated with the types of lesions caused by pathogens (Pertry et al. 2010).

A 674-kb pathogenicity island (PAI) was discovered in *S. turgidiscabies* in 2005, and it was the first PAI identified in gram-positive bacteria (Huguet-Tapia et al. 2014; Kers et al. 2005). In *S. turgidiscabies* Car8, the PAI harbors four virulence factors: the ThxA synthetic gene cluster, *nec1*, *tomA*, and the *fas* operon (Huguet-Tapia et al. 2011; Kers et al. 2005). However, in *S. scabiei* and *S. acidiscabies*, the PAI contains all of the virulence factors except the *fas* operon, and the PAI is divided into two regions, the toxin region (TR) and the colonization region (CR), which are far apart in the linear chromosome of *Streptomyces* spp. In *S. scabiei* 87-22, these two regions are 4.9 Mb apart (Lerat et al. 2009), and the TR is further separated into two subregions (TR1 and TR2) by attachment (*att*) sites (Chapleau et al. 2016). TR1 contains the ThxA biosynthetic gene cluster, and TR2 includes an integrative and conjugative element (ICE) (Chapleau et al. 2016), whereas the CR contains only the *nec1* and *tomA* genes (Lerat et al. 2009).

Horizontal gene transfer (HGT) is a method by which bacteria share genetic material, promoting bacterial evolution and enabling the rapid emergence of new pathogenic species (Eggers et al. 2016). Thus, it is speculated that the major causative genes of the scab caused by streptomycetes form ICEs for mobilization (Chapleau et al. 2016). Most ICEs are transferred as single-stranded DNA, whereas conjugative elements are transferred as double-stranded DNA in streptomycetes and related actinomycetes (Huguet-Tapia et al. 2014). ICEs are specifically excised from host chromosomes to form extrachromosomal circulatory elements, which can then be conjugated into recipient cells and specifically integrated into the chromosomes of recipient cells (Bordeleau et al. 2012; Chapleau et al. 2016). Therefore, the mobilization of PAIs is thought to be the main driving force for the evolution of phytopathogenic streptomycetes (Loria et al. 2006).

Before assessing the evolution of PAIs in pathogenic *Streptomyces* species, a clear understanding of the phylogenetic relationship between pathogenic *Streptomyces* species and nonpathogenic *Streptomyces* species is crucial; 16S ribosomal RNA (rRNA) sequence analysis is the standard method for bacterial identification (Kreuze et al. 1999; Zhang et al. 2016a), but the high similarity of the 16S rRNA sequences of *Streptomyces* spp. limits the application of this identification method for these bacteria (Labeda et al. 2012). To successfully carry out accurate identification of *Streptomyces* species, multilocus sequence analysis has been rapidly developed (Guo et al. 2008). Multiple internal fragments of various enzyme-encoding genes, such as ATP synthase F1 (beta subunit; *atpD*), DNA gyrase (beta subunit; *gyrB*), recombinase A (*recA*), RNA polymerase (beta subunit; *rpoB*), and tryptophan synthase (beta subunit; *trpB*), can be used to identify closely related *Streptomyces* species (Guo et al. 2008). In addition, specific primers for different strains can be designed based on the variable regions of the 16S rRNA gene sequences of pathogenic strains (Wanner 2006), such as *S. scabiei*, *S. europaeiscabiei*, *S. stelliscabiei*, *S. bottropensis*, *S. acidiscabies*, *S. turgidiscabies*, and *Streptomyces aureofaciens*. Therefore, polymerase chain reaction (PCR) amplification of target fragments with specific primers is used to rapidly identify and isolate pathogenic *Streptomyces* spp. (Dees et al. 2013; Leiminger et al. 2013).

In this study, the systematic pathogenicity verification and molecular identification of the pathogenic strain *Streptomyces* sp.

AMCC400023, which was isolated and stored in our laboratory, was carried out. Subsequently, whole-genome analysis was performed to determine the compositional differences and phylogenetic relationships between the test strain and related pathogenic strains. Simultaneously, the structure composition as well as the possible evolution development of gene island PAI are also discussed here at the genome level.

MATERIALS AND METHODS

Bacterial strains and culture conditions. The *Streptomyces* sp. AMCC400023 strain used in this study was isolated from potato scabs in the Dagong Village (39°31'47"N; 118°14'36"E), Daodi Town, Lunan District, Tangshan City, Hebei Province, China. All *Streptomyces* strains were grown at 28°C using oat agar (OMA) medium (International *Streptomyces* Project 3 [ISP 3]) or Gause's No. 1 medium. All liquid cultures (NA medium) were shaken at 250 rpm at 28°C.

DNA extraction. According to the method described by Abdel-Rahman et al. (2012), the AMCC400023 strain was inoculated into YEME liquid medium and cultured at 28°C for 48 h. The DNA from the *Streptomyces* strain was extracted and dissolved in TE buffer, and the purity was determined. The DNA was then stored at -20°C (Abdel-Rahman et al. 2012).

Pathogenicity-associated genes identification. The sequences of the primer pairs used for PCR are shown in Table 1. PCR amplification was conducted in a 25- μ l reaction system that contained 12.5 μ l of 2 $\times$ TransStart FastPfu Fly PCR SuperMix (TaKaRa), 1 μ l of each primer, 0.5 μ l of DNA template, and MilliQ water to bring the volume to 25 μ l. The amplified product was detected by agarose gel (1%) electrophoresis.

ThxA production. The mycelial suspensions (10 μ l) of *Streptomyces* sp. AMCC400023 and *S. scabiei* NRRL B-16523 strain were inoculated into 3 $\times$ 100 ml of OBB medium in a 250-ml flask and shaken at 28°C for 6 days (200 rpm). After culturing for 6 days, the medium was centrifuged at 8,000 rpm for 20 min, and the supernatant was collected. The supernatant was extracted twice with an equal volume of ethyl acetate, and after rotary evaporation, the residue was dissolved in 2 ml of acetonitrile to get the ThxA solution. The solution was filtered through a 0.2- μ m polytetrafluoroethylene filter and analyzed using LC-MS/MS (4000 Q TRAP; AB SCIEX USA) with a 0.4 ml/min flow rate of an isocratic mobile phase of 40:60 acetonitrile:water. ThxA was detected according to the method described previously (Leclerc et al. 2017). The mycelia of the culture strains were dried and weighed as a measure of *Streptomyces* strains. The average ThxA production of *Streptomyces* sp. AMCC400023 strain based on dry cell weight was set to 100%. The average ThxA production of *S. scabiei* NRRL B-16523 based on dry cell weight was relatively obtained from the value of *Streptomyces* sp. AMCC400023. All experiments were repeated three times with different biological samples of *Streptomyces* strains.

Tuber slice assay and radish seedling test. In the potato tuber slice assay, according to the method described by Loria et al. (1995), a healthy potato tuber slice was selected to inoculate pathogen-containing agar blocks in the dark at 25°C (Loria et al. 1995). After 10 days, the ratio of necrosis area to inoculum area was measured (Hao et al. 2009). The above test was repeated three times.

The in vitro radish seedling test was performed as described previously (Bignell et al. 2010b); however, the Burpee White variety was replaced with the South Korean variety of radish seeds. Mycelial suspensions were used to inoculate three similarly sized newly germinated radish seedlings, and an equal amount of noninoculated pathogenic culture medium was used as a blank control. The percentage of inhibition of radish sprout growth was used as an indicator of pathogenicity (Leiner et al. 1996). This experiment was repeated three times for each sample.

Potted back test of pathogenicity. Healthy potato tubers of variety Dutch 15 were selected for germination and then sown. The

spore suspension was inoculated during the expansion period so that the final concentration of the pathogenic *Streptomyces* strain in the substrate was 10^6 cfu/cm³. Each treatment was repeated in five pots. A sample that was not treated with the pathogen was used as a blank control. After 90 days of cultivation, the disease index was calculated, and DNA was extracted from the lesions for PCR-based detection. Disease index (percentage) = $\Sigma(\text{incidence level} \times \text{number of corresponding grades}) / (\text{the highest incidence level} \times \text{total number in this survey}) \times 100$ (Hao et al. 2009).

Morphological, physiological, and biochemical characteristics of pathogenic *Streptomyces* species. The morphological characteristics of pathogenic *Streptomyces* sp. AMCC400023, such as spore chains, spore color, and soluble pigments, were evaluated using the methods described in the ISP (Shirling and Gottlieb 1966). The colonies and spores were observed on OMA medium for 14 days at 14°C, and the observations were recorded.

The carbon source utilization, nitrogen source utilization, and sensitivity of the pathogenic *Streptomyces* sp. AMCC400023 were measured as described previously (Lambert and Loria 1989b; Shirling and Gottlieb 1966).

Cloning and sequencing of the 16S rRNA gene. After the DNA of *Streptomyces* sp. AMCC400023 was extracted, the 16S rDNA gene sequence was amplified using the universal *Streptomyces* primer pair PA/PH (Edwards et al. 1989). The 16S rRNA sequences were compared by BLAST, and a phylogenetic tree was constructed using the neighbor-joining algorithm with Mega7.0.14 software.

De novo genome sequencing and assembly. We sequenced whole genome of the *Streptomyces* sp. AMCC400023 strain using PacBio RSII third generation sequencing technology. For the original genomic data, the hierarchical genome assembly process algorithm was used for genome assembly. Gene discovery and annotation were performed using the Subsystems Technology (RAST) server (Aziz et al. 2008) and BLAST comparison tools.

Ortholog retrieval and single-copy cluster search. To construct a phylogenetic tree, *Brevibacillus laterosporus* AMCC100017 was used as the outgroup. The protein-coding sequences of 31 *Streptomyces* strains (Table 2) and one outgroup were used for the identification of homologous clusters by OrthoMCLv2.0.9 (Li et al. 2003). Clustering by OrthoMCL software was performed by BLAST similarity comparison with default settings. Single-copy homologous genes in the genome were selected as potential markers for phylogenetic analysis.

Gene alignment and filtration criteria. To minimize the low-quality fragments in the comparative analysis, various filtration standards were used as described previously (Zhang et al. 2016a). Multiple sequence alignments were performed for the genome of each strain using MUSCLEv3.8.31, and the calculations were compared by using default settings (Edgar 2004a, b).

Phylogenetic evaluation by the maximum likelihood method. Nucleotide sequence alignments of the genes were performed using the maximum likelihood method on the RAXMLv8.2.11 platform (Stamatakis 2014; Zhang et al. 2016a). The calculation parameters were as follows: -fa -x 12345 -p 12345 -# 100 -m PROTGAMMALGX. To estimate the reliability of the tree, 1,000 self-examination values were adopted.

Average nucleotide identity and in silico DNA-DNA hybridization analyses. Average nucleotide identity (ANI) analysis of the whole genome was performed using the methods suggested by Richter and Rosselló-Móra (2009). Paired ANI values were obtained by using nucmer (Delcher et al. 2002) with script calculate_an.py (https://github.com/widdowquinn/scripts/blob/master/bioinformatics/calculate_an.py).

In silico DNA-DNA hybridization (isDDH) was calculated using the genome-to-genome distance calculator (GGDC) (Auch et al. 2010; Meier-Kolthoff et al. 2013). The contig files were uploaded to the GGDC2.0 web server (<http://ggdc.dsmz.de/distcalc2.php>), where the isDDH calculations were performed using only Formula 2.

Gene collinearity analysis. The genomic sequences of selected major pathogens were obtained from the NCBI database. With the *Streptomyces* sp. AMCC400023 strain as a reference strain, collinearity analysis was performed by MScanXv 0.8 (Wang et al. 2012). The algorithm parameters were set to default values.

Identification of toxin genes in pathogenic *Streptomyces* species. Using the whole-genome sequences of *S. scabiei* 87-22 and *Streptomyces* sp. AMCC400023, BLAST alignments were performed with the Virulence Factors of Pathogenic Bacteria Database to identify all pathogenicity-related genes in these two pathogens.

RESULTS

Pathogenicity verification of *Streptomyces* sp. AMCC400023.

Streptomyces sp. AMCC400023 that causes potato scab was isolated from a diseased potato sample in the Dagong Village (39° 31' 47" N; 118° 14' 36"), Daodi Town, Lunan District, Tangshan City, Hebei Province, China. The potato tuber slice assay and the radish seedling test were conducted to verify the pathogenicity of this strain, and the results are shown in Figure 1. On treatment, *Streptomyces* sp. AMCC400023 exhibited similar symptoms as the positive control *S. scabiei* (type strain NRRL B-16523), and the pathogenic test results are shown in Table 3. A potted back experiment of isolate AMCC400023 was also conducted to verify the pathogenicity of this strain. Potatoes were harvested after cultivation for 90 days, and the results showed that

TABLE 1. Primer sequences used in the experiments

Amplified gene and primer	Primer sequence	Fragment size (bp)	Reference
16S rRNA ^a			
PA	5'-TTTGATCCTGGCTCAG-3'	1,535	Edwards et al. 1989
PH	5'-AAGGAGGTGATCCAGCCGCA-3'		
16S-23S ITS			
ITS-L	5'-GTCAAGTCATCATGCCCTT-3'	650	Song et al. 2004
ITS-R	5'-AAACTTGGCCACAGATGCTC-3'		
txtAB			
txtAB1	5'-CCACCAGGACCTGCTCTTC-3'	385	Wanner 2006
txtAB2	5'-TCGAGTGGACCTCACAGATG-3'		
nec1			
Nf	5'-ATGAGCGCGAACGGAAGCCCCGGA-3'	700	Bukhalid et al. 2002
Nr	5'-GCAGGTCGTACGAAGGATCG-3'		
tomA			
Tom3	5'-GAGGCGTTGGTGGAGTTCTA-3'	392	Wanner 2006
Tom4	5'-TTGGGGTTGTACTCCTCGTC-3'		

^a rRNA, ribosomal RNA.

inoculation with the AMCC400023 strain caused typical scab lesions on the potato surfaces (Fig. 11) with an incidence rate of 42.9% (Table 3).

ThxA production by *Streptomyces* sp. AMCC400023 and *S. scabiei* NRRL B-16523 was analyzed in OBB medium, which is a complex medium for inducing ThxA biosynthesis (Wach et al. 2007). The two test strains were able to synthesize ThxA as expected by LC-MS/MS analysis (Fig. 2), whereas the amount of ThxA synthesized by *S. scabiei* NRRL B-16523 strain was significantly higher than that by *Streptomyces* sp. AMCC400023.

Morphological, physiological, and biochemical characteristics of *Streptomyces* sp. AMCC400023. *Streptomyces* sp. AMCC400023 was cultured in OMA and ISP medium at 28°C for 14 days. After being cultured for 14 days, *Streptomyces* sp. AMCC400023 formed abundant endophytic and aerial hypha. The mature spores were gray and columnar, and the spore chains were loose spiral. The aerial hypha were gray to white in different media, and the substrate mycelium was gray to yellow or yellowish brown on different media. Soluble pigments were produced on ISP 6 medium. *Streptomyces* sp. AMCC400023 produced melanin on tyrosine medium and could utilize D-glucose, D-mannitol, raffinose, rhamnose, sucrose, D-xylose, inositol, and other sugars. In addition, L-histidine and L-methionine in nitrogen source could also be utilized by this strain. The specific characteristics of the colony morphology, microscopic morphology, and scanning electron micrographs are shown in Figure 3 and Table 4, and the physiological and biochemical characteristic results of *Streptomyces* sp. AMCC400023 compared with those of related pathogenic streptomycetes from other studies are shown in Table 5.

16S rRNA phylogenetic tree of *Streptomyces* sp. AMCC400023 and related pathogenic streptomycetes. After DNA extraction, the 16S rDNA gene sequence of *Streptomyces* sp. AMCC400023 was

amplified by PCR and sequenced (GenBank accession number KU189257). Subsequently, whole-genome sequencing of this strain was performed, and a full sequence was obtained. There were six 16S rRNA gene sequences in the genome, and one of them was chosen to construct a phylogenetic tree. The phylogenetic tree showed that the 16S rRNA gene sequence amplified by 16S rDNA and the 16S rRNA gene sequences present in the genome of the AMCC400023 strain were closely related to the type species *S. bottropensis* ATCC25435 (Fig. 4). Considering the complicated situation in the identification of streptomycetes, more work should be carried out to obtain the accurate taxonomic status.

Phylogenetic analysis and genomic similarity analysis between pathogenic and nonpathogenic *Streptomyces* species. To accurately predict the phylogenetic relationships of strain AMCC400023 (GenBank complete genome accession number CP024989) with those species among the genus *Streptomyces*, 31 *Streptomyces* strains, including pathogenic and nonpathogenic species, were selected from the NCBI database for comprehensive phylogenetic analysis. A total of 1,104 single-copy homologous gene clusters from these 31 strains and 1 outgroup strain (*B. laterosporus* AMCC100017) were first screened using OrthoMCLv2.0.9 (Li et al. 2003). The nucleotide sequences of these 1,104 single-copy gene groups were matched to eliminate mismatched sites, and 500 orthologous groups remained. The remaining genes were subjected to linkage analysis, and the maximum likelihood method was used to predict the phylogenetic relationships among the 31 *Streptomyces* strains.

In the phylogenetic tree in Figure 5, pathogenic *Streptomyces* strains were divided into three major branches. The main pathogen *S. acidiscabies* represented one branch, and *S. turgidiscabies* represented one branch and was located in the farthest position. Interestingly, *S. scabiei*, *S. europaeiscabiei*, *S. bottropensis*

TABLE 2. Genomic statistical data of pathogenic and nonpathogenic *Streptomyces* species

Species	Strain name	Genome status	Contig number	Contig N50* (kb)	Genome size (Mb)	Geographic location	GenBank assembly accession number	Source or reference
<i>Streptomyces</i> sp.	AMCC400023	Complete			10.7	Hebei, China	CP024989	This article
<i>S. bottropensis</i>	ATCC25435	Draft	109	195.2	8.9	Unknown	AOCF00000000.1	
<i>S. europaeiscabiei</i>	96-14	Draft	931	21.2	10.5	Australia	GCA_001550375.1	Zhang et al. 2016a
<i>S. europaeiscabiei</i>	89-04	Draft	1,080	17.7	10.5	Alaska, U.S.A.	GCA_001550325.1	Zhang et al. 2016a
<i>S. europaeiscabiei</i>	NRRL B-24443	Draft	1,056	16.4	10	Le Rhue, France	GCA_000988945.1	Labeda et al. 2012
<i>S. stelliscabiei</i>	NRRL B-24447	Draft	991	19.0	10.0	France	GCA_001008135.1	Labeda et al. 2012
<i>S. scabiei</i>	87-22	Complete			10.1	Wisconsin, U.S.A.	GCA_000091305.1	Bignell et al. 2010a, b
<i>S. scabiei</i>	95-18	Draft	1,798	10.6	10.5	South Africa	GCA_001550225.1	Zhang et al. 2016a
<i>S. scabiei</i>	84-34	Draft	1,650	10.9	10.0	Upstate New York, U.S.A.	GCA_001550245.1	Zhang et al. 2016a
<i>S. scabiei</i>	NRRL B-16523	Draft	1,091	15.1	9.8	Central New York, U.S.A.	GCA_001005405.1	Labeda et al. 2012
<i>S. scabiei</i>	84-232	Draft	1,464	12.6	9.8	West Virginia, U.S.A.	GCA_001572115.1	Zhang et al. 2016a
<i>S. scabiei</i>	96-08	Draft	1,374	10.6	11.2	Alberta, Canada	GCA_001550235.1	Zhang et al. 2016a
<i>S. scabiei</i>	NRRL B-24093	Draft	1,675	11.5	9.9	Egypt	GCA_002155765.1	Labeda et al. 2012
<i>S. scabiei</i>	NRRL B-2801	Draft	1,521	14.1	10.3	Presque Isle, ME, U.S.A.	GCA_002155725.1	Labeda et al. 2012
<i>S. scabiei</i>	96-06	Draft	1,173	9.9	9.0	Ontario, Canada	GCA_001579685.1	Zhang et al. 2016a
<i>Streptomyces</i> sp.	96-12	Draft	1,875	8.7	10.1	Egypt	GCA_001550315.1	Zhang et al. 2016a
<i>S. ipomoeae</i>	91-03	Draft	687	26.4	10.4	U.S.A.	GCA_000317595.1	Huguet-Tapia et al. 2014
<i>S. acidiscabies</i>	84-104	Draft	244	113.4	11.0	Upstate New York, U.S.A.	GCA_000242715.2	Huguet-Tapia et al. 2011
<i>S. acidiscabies</i>	98-48	Draft	983	20.3	10.6	Saga, Japan	LYDS00000000	Zhang et al. 2016b
<i>S. acidiscabies</i>	98-49	Draft	1,211	16.5	10.2	Saga, Japan	LYDT00000000	Zhang et al. 2016b
<i>S. niveiscabiei</i>	NRRL B-24457	Draft	1,568	10.1	9.5	Jeju, South Korea	GCA_001419795.1	Labeda et al. 2012
<i>S. diastatochromogenes</i>	ATCC 12309	Draft	1,280	18.6	12.3	Japan	GCA_001550305.1	Zhang et al. 2016a
<i>S. galbus</i>	KCCM 41354	Draft	104	24.2	10.1	Korea	GCA_000772895.1	
<i>S. coelicolor</i>	A3(2)	Complete			9.1	England	GCA_000203835.1	
<i>S. turgidiscabies</i>	Car8	Draft	692	27.5	10.8	Japan	GCA_000331005.1	Huguet-Tapia et al. 2011

* Contig N50 defines the quality of the sketch assembly. N50 refers to the length of the last contig or scaffolding when the assembled contig or scaffolds are arranged in descending order and their cumulative length just exceeds 50% of the total length of the entire assembly sequence. For the complete genome, no N50 is available.

ATCC25435, and *Streptomyces* sp. AMCC400023, each representing a small branch, were clustered into one large branch. The test strain *Streptomyces* sp. AMCC400023 exhibited a relatively close relationship with *S. scabiei* in the phylogenetic tree.

ANI and isDDH analyses among *Streptomyces* phytopathogens at the genomic level. The ANI and isDDH values and core genome-based phylogenetic analysis showed that *Streptomyces* sp. AMCC400023 was clustered with the *Streptomyces* sp. 96-12 and *S. scabiei* NRRL B-24093 strains instead of with the *S. bottropensis* ATCC25435 strain, although the similarity between the 16S rRNA sequences of *Streptomyces* sp.

AMCC400023 and *S. bottropensis* ATCC25435 reached 100%. At the same time, *Streptomyces* sp. AMCC400023 could not be classified into a member of *S. scabiei* owing to its ANI and isDDH values as well as the genome core tree in Figure 6. This result indicated that *Streptomyces* sp. AMCC400023 might be a distinct pathogenic *Streptomyces* species.

PAI structure and composition of *Streptomyces* sp. AMCC400023. Gene composition analysis of *Streptomyces* sp. AMCC400023 PAI was performed by a hidden Markov model (Punta et al. 2011), and a PAI structure plot was then constructed (Fig. 7). The results showed that TR1 contained 10 open reading

Fig. 1. Pathogenicity verification of *Streptomyces* sp. AMCC400023. **A, B, and C,** Potato tuber slice assay, **D, E, and F,** radish seedling test, and **G, H, and I,** potted back experiment. **A, D, and G,** Blank control without pathogen inoculation. **B, E, and H,** Inoculated with positive control *Streptomyces scabiei* NRRL B-16523. **C, F, and I,** Inoculated with *Streptomyces* sp. AMCC400023.

TABLE 3. Pathogenic test results of *Streptomyces* sp. AMCC400023

Strain	Potato tuber slice assay, %	Radish seedling inhibition rate, %	Pot experiment disease index, %
<i>S. scabiei</i> NRRL B-16523	100	88.2	42.9
<i>Streptomyces</i> sp. AMCC400023	96	84.7	37.5

frames (ORFs). These 10 ORFs included the thaxtomin biosynthetic gene cluster and other genes encoding putative proteins, whereas there were no genes associated with ICEs in this region. TR2 contained 151 ORFs, including lantibiotic synthesis gene clusters, site-specific integrases, and recombination directionality factors. Related genes for conjugative transfer were present in this region, and a locus with replication function was also identified in this region. There were 80 ORFs in the CR region, including the *nec1* gene, which encoded a necrotic protein, and the *tomA* gene, which encoded

tomatinase. In this region, there were many genes that were involved in sugar metabolism. However, the gene encoding the IS110 family transposase was deleted in TR2 of *Streptomyces* sp. AMCC400023. We also found that the *Streptomyces* sp. AMCC400023 strain contained genes encoding NADP oxidoreductase, an HxIR family transcriptional regulator, and the peptidase S12 upstream of the CR. These three genes were not found in *S. scabiei*.

Phylogenetic comparison of the ThxA biosynthetic gene cluster and the core genome. The TR in the PAI, containing the

Fig. 2. LC-MS/MS analysis of thaxtomin A extracts of *Streptomyces* strains. **A**, The secondary mass spectrum of thaxtomin A was analyzed by LC-MS/MS. **B**, Production of thaxtomin A by *Streptomyces* sp. AMCC400023 and *Streptomyces scabiei* NRRL B-16523. The average thaxtomin A production of *Streptomyces* sp. AMCC400023 strain based on dry cell weight was set to 100%. The average thaxtomin A production of *S. scabiei* NRRL B-16523 based on dry cell weight was relatively obtained from the value of *Streptomyces* sp. AMCC400023. The error bars represented the standard deviations of the mean.

ThxA synthetic gene cluster, could participate in conjugative transfer, leading to the formation of novel *Streptomyces* spp. To explore the evolutionary origin of the PAI in *Streptomyces* sp. AMCC400023, the phylogenetic tree of ThxA was constructed using *txtA*, *txtB*, *txtC*, *txtD*, *txtE*, *txtH*, and *txtR* from the ThxA biosynthetic gene cluster (Fig. 6). In the ThxA phylogenetic tree, *Streptomyces* sp. AMCC400023 clustered closely with *S. acidiscabies* and *S. scabiei*, indicating that the ThxA synthetic gene cluster in the genomes of these strains was highly similar and homologous. In contrast, these strains were far apart in the tree for the core genome. Therefore, we hypothesized that *Streptomyces* species *Streptomyces* sp. AMCC400023, *S. acidiscabies*, and *S. scabiei* harbored the same ThxA synthetic gene cluster and that they all had the same evolutionary origin and were disseminated by HGT. Although *S. europaeiscabiei* and *S. stelliscabiei* harbored the same ThxA synthetic gene cluster, *S. turgidiscabiei* was located separately in a far position.

Collinearity analysis of different pathogenic *Streptomyces* species. In the collinearity analysis, *Streptomyces* sp. AMCC400023 was used as a reference strain, and the main and well-studied pathogens *S. scabiei* 87-22, *S. scabiei* NRRL B-24093, type strain *S. scabiei* NRRL B-16523, *S. scabiei* NRRL B-2801, *S. acidiscabies* 84-104, *S. turgidiscabiei* Car8, and *S. europaeiscabiei* NRRL B-24443 were selected for this analysis (Fig. 8).

In the collinearity map, *Streptomyces* sp. AMCC400023 shared a large number of similar genes with those related pathogenic streptomycetes, and most of them were genes located in PAI. Consensus genes between *Streptomyces* sp. AMCC400023 and *S. turgidiscabiei* Car8 as well as *S. europaeiscabiei* NRRL B-24443 all located in the area of 0 to 5 M bp of genome size, whereas the consensus genes between *Streptomyces* sp. AMCC400023 and *S. acidiscabies* 84-104, type strain *S. scabiei* NRRL B-16523, *S. scabiei* NRRL B-24093, and *S. scabiei* NRRL B-2801, all located near the position of 0, 3, and 10 M of genome size. Interestingly, consensus sequence between *Streptomyces* sp. AMCC400023 and *S. scabiei* 87-22 was very dense, which indicated a similar and close gene arrangement. These homologous sequences or in another words, these homologous genes were a clear gene transfer proof, although we could not distinguish the exact gene transfer direction.

Toxicity-associated gene analysis between *Streptomyces* sp. AMCC400023 and *S. scabiei* 87-22. A comparative genomic analysis in the Virulence Factors of Pathogenic Bacteria Database was performed between *Streptomyces* sp. AMCC400023 and the well-studied *S. scabiei* 87-22. In this analysis, besides 69 common toxicity-associated genes, 60 unique virulence-associated genes were verified in the genome of *Streptomyces* sp. AMCC400023, and 58 unique toxicity-associated genes were found in *S. scabiei* 87-22 genome, all of which were associated with secreted proteins,

enzymes, etc. These results showed that there were certain differences in genetic composition between *Streptomyces* sp. AMCC400023 and *S. scabiei* 87-22. In addition, this analysis identified 69 common virulence-associated genes in the *Streptomyces* sp. AMCC400023 genome, most of which encoded proteins, such as putative secretory proteins, transcriptional regulators, membrane transporters, and enzymes involved in secondary metabolite biosynthesis, and the transcription of these genes might enhance pathogenicity.

In addition, the *Streptomyces* sp. AMCC400023 genome contained certain genes in the coronatine (COR) synthetic gene cluster that had been identified in *S. scabiei*. COR is a major virulence factor in the gram-negative plant pathogen *Pseudomonas syringae*, and it causes various symptoms in plant hosts, such as chlorosis, inhibition of root growth, and stimulation of ethylene synthesis (Bender et al. 1999; Bignell et al. 2010b). The *Streptomyces* sp. AMCC400023 genome also contained genes associated with the synthesis of the plant hormone indole-3-acetic acid (IAA), which indicated that the organism could produce IAA from L-tryptophan via the indole-3-acetamide pathway (McClerklin et al. 2018; Spaepen et al. 2007). Other than the two synthetic pathways above, the *Streptomyces* sp. AMCC400023 strain also contained cutinase and other enzymes that might be involved in successful infection of host plants. All of these data supported that *Streptomyces* sp. AMCC400023 was different from *S. scabiei*, and there was enough evidence to show that it was a distinct phytopathogen that causes potato common scab.

DISCUSSION

Streptomyces sp. AMCC400023 was isolated in our laboratory from infected potato scabs in Hebei Province, China. This strain was first identified as *S. bottropensis* based on morphological,

TABLE 4. Growth characteristics of *Streptomyces* sp. AMCC400023 on different mediums^a

Medium	Aerial mycelia	Substrate mycelium	Soluble pigment
Tryptone-yeast extraction (ISP 1)	W	Y	No
Yeast-malt extract agar (ISP 2)	B	B	No
Oat medium (ISP 3)	G	YW	No
Inorganic salt-starch agar (ISP 4)	G	YG	No
Glycerol-asparagine agar (ISP 5)	B	B	Y
Peptone-yeast extract iron agar (ISP 6)	GW	LBB	B
Tyrosine agar (ISP 7)	YW	Y	No
Nutrient agar	W	W	No

^a B, brown; G, gray; GW, grayish white; ISP, International *Streptomyces* Project; LBB, light black brown; W, white; Y, yellow; YG, yellowish gray; and YW, yellowish white.

Fig. 3. Morphological characteristics of the strain *Streptomyces* sp. AMCC400023. A, Colony morphology on oat agar medium for 14 days. B, Microscopic morphology of aerial mycelium (400 $\times$). C, Spore-bearing mycelium under a scanning electron microscope.

physiological, and biochemical characteristics as well as 16S rDNA analysis (Zhou et al. 2016). In this paper, after verifying the pathogenicity of *Streptomyces* sp. AMCC400023, systematic analyses, including morphological, physiological, and biochemical characterization; 16S rRNA gene analysis; and genomic ANI and isDDH analyses, were carried out. Compared with data from DDH analysis, isDDH data are reproducible and have direct utility (Goris et al. 2007), and ANI analysis is also provides a good estimate of evolutionary distance (Zhang and Qiu 2015, 2016; Zhang et al. 2016b). In general, the ANI value 95%, which is equivalent to the isDDH value 70%, can be used for species identification (Goris et al. 2007). The complete genome analysis based on the NCBI database (Fig. 5) showed that *Streptomyces* sp. AMCC400023 was

clustered with *Streptomyces* sp. 96-12, *S. scabiei* NRRL B-24093, and *S. scabiei* NRRL B-2801 in the phylogenetic tree, which was completely consistent with ANI and isDDH results (Fig. 9). According to a genome-wide phylogenetic tree and ANI and isDDH analysis, *S. scabiei* NRRL B-24093 and *S. scabiei* NRRL B-2801 were likely to be misidentified as *S. scabiei*. In fact, *Streptomyces* sp. AMCC400023, strain NRRL B-24093, and strain NRRL B-2801 were an independent species differing from *S. scabiei* under the genus *Streptomyces*. The spores formed by *Streptomyces* sp. AMCC400023 were gray and columnar, and the spore chains were loosely spiral. Rich in endophytic hyphae and aerial hyphae, the aerial hyphae were gray to white in different media, and the hyphae in the base were gray to yellow or yellowish brown. Soluble

TABLE 5. Comparison of physiological and biochemical characteristics between *Streptomyces* sp. AMCC400023 and related potato scab pathogens^a

Characteristic index	AMCC400023	<i>S. scabiei</i>	<i>S. turgidiscabies</i>	<i>S. acidiscabies</i>	<i>S. caviscabies</i>	<i>S. stelliscabiei</i>	<i>S. europaeiscabiei</i>
Spore color	G	G	G	W	W	G	G
Spore chain	Sp	Sp	Rf	Rf	Rf	Sp	Sp
Melanin	+	+	–	–	–	+	+
D-Fructose	–	+	+	+	–	+	+
D-Glucose	+	+	–	ND	ND	+	+
D-Mannitol	+	+	+	+	–	+	+
Raffinose	+	+	+	–	+	+	+
Rhamnose	+	+	+	+	–	+	+
Sucrose	+	+	+	+	–	+	+
D-Xylose	+	+	+	+	–	+	+
Inositol	+	+	+	+	ND	+	+
L-Histidine	+	+	+	+	ND	ND	ND
Methionine	+	+	+	+	+	ND	ND
pH 4.0	–	–	–	+	ND	ND	ND
pH 4.5	+	–	+	+	–	ND	ND
pH 5.0	+	+	+	+	ND	ND	ND
5.0% NaCl	+	+	–	ND	ND	–	ND
Crystal violet (0.5 µg/ml)	+	–	–	+	V	–	–
Phenol (0.1%)	+	–	–	+	+	ND	ND
Penicillin (10 IU/ml)	+	–	+	+	+	+	+
Streptomycin (20 µg/ml)	+	–	–	+	+	–	–

^a *S. scabiei* (Lambert and Loria 1989b), *S. turgidiscabies* (Miyajima et al. 1998), *S. acidiscabies* (Lambert and Loria 1989a), *S. caviscabies* (Goyer et al. 1996), and *S. stelliscabiei* and *S. europaeiscabiei* (Bouchek-Mechiche et al. 2000). A positive reaction is indicated by +, and a negative reaction is indicated by –. G, gray; ND, uncertain; Rf, straight flexible; Sp, spiral; V, according to strain differences; and W, white.

Fig. 4. Phylogenetic analysis for 16S ribosomal RNA (rRNA) genes of *Streptomyces* sp. AMCC400023 and related pathogenic streptomycetes. 16S rRNA from the genome means that it was from genome analyses. 16S rRNA from polymerase chain reaction (PCR) means that it was obtained by PCR amplification of our extracted DNA.

pigments were produced on ISP 6 medium. As for the comparison of physiological and biochemical characteristics between *Streptomyces* sp. AMCC400023 and *S. scabiei*, test strain *Streptomyces* sp. AMCC400023 could utilize D-fructose, survive at pH 4.5, and grow at crystal violet (0.5 µg/ml), phenol (0.1%), penicillin (10 IU/ml), and streptomycin (20 µg/ml), all differed from *S. scabiei*. More important, there were certain differences in virulence-concerned

genetic composition between *Streptomyces* sp. AMCC400023 and *S. scabiei* 87-22. The genome sequence of type strain *S. scabiei* NRRL B-16523 in NCBI could provide enough evidence to help us obtain the final identification results of *Streptomyces* sp. AMCC400023. Combining all of the analyses results above, we concluded that *Streptomyces* sp. AMCC400023 was a completely unique phytopathogenic species causing potato common scab. Of

Fig. 5. *Streptomyces* phylogenetic tree among 31 *Streptomyces* species using the maximum likelihood algorithm RAxML based on the selection of 500 single-copy gene clusters in the *Streptomyces* genomes. A total of 1,000 bootstraps were calculated. The number above the branch is the percentage of the bootstrap value.

all of the methods used for the identification of *Streptomyces* species, the genome-level data analyses were the most valuable and persuasive methods for *Streptomyces* species identification. Our result was consistent with the opinion that single-gene phylogenetic analysis does not solve the problems associated with streptomycete identification (Zhang et al. 2016a), and genome-based analysis tools could facilitate accurate identification of this unique genus (Auch et al. 2010; Richter and Rosselló-móra 2009).

Currently, the development of sequencing technology has provided us with >30 genomic sequences of pathogenic *Streptomyces* strains (Zhang et al. 2016a). Simultaneously, the development of comparative genomics has helped accurately identify pathogenic factors from different pathogenic isolates. Zhang et al. (2016a) analyzed the PAI composition of some important pathogens, including not only different pathogenic isolates, but also different strains belonging to the same species (Zhang and Loria 2017; Zhang et al. 2016a). In this paper, the complete PAI composition of *Streptomyces* sp. AMCC400023 was identified, and the PAI was found to contain three genes encoding virulence factors, namely a ThxA synthetic gene cluster, *nec1*, and *tomA*; the PAI of this strain was seen to be divided into two regions (TR and CR), where the TR included a ThxA synthetic gene cluster with the genes *txtA*, *txtB*, *txtC*, *txtD*, *txtE*, *txtH*,

and *txtR*. *txtA* and *txtB* encode nonribosomal peptide synthetases (NRPSs), *txtC* encodes a P450 monooxygenase, *txtD* encodes a nitric oxide synthase, and *txtE* encodes a cytochrome P450 enzyme responsible for tryptophan-specific nitrification (Bignell et al. 2014). *txtR* encodes an AraC family transcriptional regulator that regulates the synthesis of ThxA in vivo (Bignell et al. 2014; Joshi et al. 2007b). *txtH* encodes a short 65-amino acid protein (Zhang et al. 2016a) that might belong to the MbtH-like protein family and play an important role in the regulation of NRPS activity (Herbst et al. 2013; Stegmann et al. 2006); however, the specific role of this protein in the synthesis of ThxA is unknown (Bignell et al. 2010a). The CR is made up of the *nec1* and *tomA* genes. The main PAI composition of *Streptomyces* sp. AMCC400023 was very similar to that of *S. scabiei* and *S. acidiscabies* reported by Lerat et al. (2009).

However, there were differences in PAI composition among different pathogenic isolates and different strains of the same genus as described previously (Zhang and Loria 2017). For example, in *S. turgidiscabies*, the PAI also harbored the *fas* operon (Hugué-Tapia et al. 2011; Kers et al. 2005). *S. acidiscabies* isolates have three different types of PAI components, namely TR1 alone (84–104), the complete TR element (a10), and no TR (98–48) (Zhang and Loria 2017). Chapleau et al. (2016) studied the mechanism for

Fig. 6. Comparison of phylogenetic analysis of phytopathogens *Streptomyces* species. Core genomes tree (right) and thaxtomins A (ThxA) synthetic gene clusters tree (left).

Fig. 7. Pathogenicity island gene structure map of *Streptomyces* sp. AMCC400023. The positions and directions of the open reading frames are shown by the arrows. The red arrows represent the thaxtomins synthetic genes, the green arrows represent the integrase and recombination directional factor (RDF), the orange arrow represents the lantibiotic synthesis genes, the blue arrow represents the conjugated integration genes, the brown arrow represents the *aviX1* gene, the light blue arrows represent *bacA* genes, the black arrow represents the *nec1* gene, the gray arrows represent the sugar metabolism genes, and the purple arrow represents the *tomA* gene. The *att* sites used to distinguish toxin region (TR; TR1 and TR2) are also shown. CR, colonization region.

the excision and integration of TR, which involved three forms of mobilization: TR1 alone, TR2 alone, and the complete TR. Although the frequency of mobilization in the TR was very low, integration of the TR or TR2 alone could be detected in the recipient strain; however, TR1 could not be excised in the test strain that lacked TR2 (Chapleau et al. 2016). Zhang et al. (2016a) found that TR2 alone and the complete TR could be transferred to the soil saprophytic bacterium *S. diastatochromogenes* ATCC 12309; integration of the complete TR conferred a pathogenic phenotype to *S. diastatochromogenes*, whereas integration of TR2 alone did not (Zhang et al. 2016a). Subsequently, Zhang et al. (2016a) conducted a detailed analysis of the transfer of the TR, but in the entire study, no individual mobilization of TR1 was observed. This

finding suggested that TR1 might be a *cis*-mobilization element that hijacked TR2 for conjugative integration, leading to HGT of pathogenic genes and the emergence of new pathogenic species (Zhang and Loria 2017).

Then, we performed a comparative genomic analysis of the PAI of *Streptomyces* sp. AMCC400023 and major scab-causing pathogens to construct a phylogenetic tree to reveal the PAI mobilization pathway in *Streptomyces* spp. Our ThxA phylogenetic analysis showed that the ThxA synthetic gene cluster of *Streptomyces* sp. AMCC400023 was highly similar to that of *S. scabiei* and *S. acidiscabies* and different from *S. europaeiscabiei*, especially *S. turgidiscabies*. Furthermore, the PAI gene composition of *Streptomyces* sp. AMCC400023 was similar to that of

Fig. 8. Genome collinearity analysis of the main pathogenic *Streptomyces* genus. Green represents *S. scabiei* NRRL B-2801, light green represents *S. scabiei* NRRL B-24093, yellow represents *S. scabiei* NRRL B-16523, orange yellow represents *S. scabiei* 87-22, orange represents *S. europaeiscabiei* NRRL B-24443, red represents *S. acidiscabies* 84-104, blue represents *S. turgidiscabies* Car8, and grass green represents *Streptomyces* sp. AMCC400023.

S. scabiei 87-22, and only some individual genes were unique in our study. To further investigate the genetic composition and evolutionary pathway of PAI, a collinearity analysis of the genomes of the main pathogenic *Streptomyces* species was performed in this study. Collinearity analysis generally provides information regarding the genetic linkages between species. During mobilization and evolution of the PAIs of pathogenic *Streptomyces* species, some genes, such as the ThxA synthetic gene cluster and the *nec1* and *tomA* genes, always maintained a linkage relationship, indicating that these genes had certain advantages during selection under certain conditions. This kind of finding provided useful clues for studying the evolution of PAIs. The results of the genomic collinearity analysis, shown in Figure 8, indicated that most genes in the genome of *Streptomyces* sp. AMCC400023 had homologous genes in the *S. scabiei* genomes, but there remained some genes that lacked corresponding homologous genes, indicating that there were some differences between the genomes of *Streptomyces* sp.

AMCC400023 and *S. scabiei*. This result was consistent with the results of the genome-wide phylogenetic analysis shown in Figure 5. In fact, the homologous genes between *Streptomyces* sp. AMCC400023 and *S. scabiei* 87-22 were so dense that we could confirm that, other than a very close relationship, there must be a gene transfer event behind between the two strains. After all, ancient *S. scabiei* was first found and studied in the scab pathogen history. In general, all of the results of the PAI composition analysis, ThxA phylogenetic analysis, genomic collinearity analysis, and ANI and isDDH analyses indicated that the PAI of *Streptomyces* sp. AMCC400023 might have been directly or indirectly acquired from *S. scabiei* by HGT.

Because of the availability of the whole-genome sequence of *S. scabiei* 87-22, Loria et al. (1997) performed a comparative genomic analysis using the *S. scabiei* 87-22 genome sequence, revealing the presence of a large number of toxicity-associated genes or gene clusters in *S. scabiei* (Bignell et al. 2010a). A majority of these genes

	<i>S. sp. AMCC400023</i>	<i>S. sp. 96-12</i>	<i>S. scabiei</i> NRRL B-24093	<i>S. scabiei</i> 87.22	<i>S. scabiei</i> NRRL B-16523	<i>S. scabiei</i> 96-06	<i>S. scabiei</i> 84-232	<i>S. scabiei</i> NRRL B-2801	<i>S. turgidiscabies</i> Car8	<i>S. turgidiscabies</i> T45	<i>S. reticuliscabies</i> NRRL B-24446	<i>S. europaeiscabies</i> 96-14	<i>S. europaeiscabies</i> NRRL B-24443	<i>S. galbus</i> KCCM 41354	<i>S. bottropensis</i> ATCC 25435	<i>S. ipomoeae</i> 91-03	<i>S. acidiscabies</i> 84-104	<i>S. coelicolor</i> A3(2)	<i>S. diastatochromogenes</i> ATCC 12309	<i>S. niveiscabies</i> NRRL B-24457	<i>S. puniscabies</i> NRRL B-24456	<i>S. stelliscabies</i> NRRL B-24447
<i>S. sp. AMCC400023</i>		83	83	37	37	38	38	72	26	26	25	38	38	46	36	31	25	25	26	25	25	38
<i>S. sp. 96-12</i>	99		99	37	5	38	38	72	27	26	26	38	39	48	38	32	25	26	26	25	26	38
<i>S. scabiei</i> NRRL B-24093	99	99		37	4	38	38	72	26	26	26	38	38	48	37	32	24	26	27	25	26	38
<i>S. scabiei</i> 87.22	88	89	89		96	96	96	36	82	25	25	39	39	35	43	31	25	25	26	25	25	45
<i>S. scabiei</i> NRRL B-16523	88	89	89	99		97	98	2	82	0	0	9	40	36	44	31	25	25	0	0	0	46
<i>S. scabiei</i> 96-06	89	89	89	99	99		98	37	82	27	82	41	41	37	45	32	26	26	26	25	26	47
<i>S. scabiei</i> 84-232	89	89	89	99	99	99		37	82	26	82	40	40	36	44	31	25	26	26	25	25	45
<i>S. scabiei</i> NRRL B-2801	96	96	96	88	88	88	88		82	82	82	89	89	92	88	86	80	81	26	25	26	38
<i>S. turgidiscabies</i> Car8	82	82	82	26	26	27	27	26		99	99	27	26	25	25	26	24	25	26	24	25	27
<i>S. turgidiscabies</i> T45	82	82	82	82	82	82	82	26	99		99	82	26	25	25	25	24	24	26	24	25	26
<i>S. reticuliscabies</i> NRRL B-24446	82	82	82	81	82	26	26	26	99	99		26	25	25	25	26	24	25	26	24	25	26
<i>S. europaeiscabies</i> 96-14	89	89	89	89	89	89	89	38	82	26	82		95	38	42	32	25	25	27	25	25	43
<i>S. europaeiscabies</i> NRRL B-24443	89	89	89	89	89	89	89	38	82	82	82	99		38	42	32	25	25	26	25	25	43
<i>S. galbus</i> KCCM 41354	92	92	92	88	88	88	88	48	81	81	82	89	89		36	32	24	25	26	24	25	37
<i>S. bottropensis</i> ATCC 25435	88	89	89	91	91	91	91	37	82	82	82	90	91	88		31	24	25	26	24	25	72
<i>S. ipomoeae</i> 91-03	85	86	86	85	85	86	86	32	82	82	82	86	86	86	85		25	26	27	25	26	32
<i>S. acidiscabies</i> 84-104	80	80	80	80	80	81	81	24	80	80	80	81	80	80	80	80		24	24	51	24	25
<i>S. coelicolor</i> A3(2)	81	82	81	81	81	82	82	26	81	81	81	81	81	81	81	81	80		25	25	26	25
<i>S. diastatochromogenes</i> ATCC 12309	82	82	82	81	85	82	82	82	82	82	82	82	82	82	82	82	80	81		25	26	26
<i>S. niveiscabies</i> NRRL B-24457	80	81	81	80	80	81	81	81	80	80	80	80	80	80	80	80	93	80	80		25	25
<i>S. puniscabies</i> NRRL B-24456	81	82	82	81	81	82	82	82	81	81	81	81	81	81	81	82	80	82	82	81		25
<i>S. stelliscabies</i> NRRL B-24447	89	89	89	91	92	92	91	89	82	82	82	90	90	89	96	86	80	81	82	81	81	

Fig. 9. The average nucleotide identity (ANI) and in silico DNA-DNA hybridization (isDDH) values of phytopathogenic and closely related nonpathogenic *Streptomyces*. The lower triangles display ANI values, and the upper triangles display isDDH values. The values that met the request of both ANI $\geq$ 99% and isDDH $\geq$ 79% are colored red, and the values among $95 \leq$ ANI $<$ 99% and $70 \leq$ isDDH $<$ 79% are colored orange.

were not detectable in other pathogenic *Streptomyces* species, but they were associated with the pathogenicity of the strain or plant–microbe interactions. This finding suggested that the pathogenicity of *S. scabiei* strains might also involve multiple toxicity determinants (Bignell et al. 2010a). Based on the availability of *Streptomyces* genomic sequences in the NCBI database, Loria et al. (1997) also conducted a comparative genomic analysis to identify toxicity-related factors that did not exist on the PAI, such as the PR-1 and CFA cluster that was conserved in *S. scabiei* (Bignell et al. 2010a). Therefore, by comparative genomic analysis of the toxin-associated genes of *S. scabiei* 87-22 and *Streptomyces* sp. AMCC400023, a total of 147 toxicity-related genes were identified. In addition to 69 common genes, *Streptomyces* sp. AMCC400023 harbored 60 unique toxicity-associated genes, and *S. scabiei* 87-22 had 58 unique toxicity-associated genes, indicating that, in addition to the PAI, the two strains also differed in their toxicity-associated gene compositions.

Based on the results of all of the genomic analyses described above, we concluded that *Streptomyces* sp. AMCC400023 was a distinct pathogenic species. The PAI of *Streptomyces* sp. AMCC400023 might have been acquired from *S. scabiei*, which was the most ancient pathogenic *Streptomyces* species causing common scab.

LITERATURE CITED

- Abdel-Rahman, T. M. A., Khalil, M. S., Moussa, T. A. A., and Al-Qaysi, A. D. A. 2012. Identification and characterization of *Streptomyces alkaliscaabies* sp. nov. J. Food Agric. Environ. 10:476-483.
- Auch, A. F., Jan, M., Klenk, H. P., and Göker, M. 2010. Digital DNA-DNA hybridization for microbial species delineation by means of genome-to-genome sequence comparison. Stand. Genomic Sci. 2:117-134.
- Aziz, R. K., Daniela, B., Best, A. A., Matthew, D. J., Terrence, D., Edwards, R. A., Formis, K., Gerdes, S., Glass, E. M., Kubal, M., Meyer, F., Olsen, G. J., Olson, R., Osterman, A. L., Overbeek, R. A., McNeil, L. K., Paarmann, D., Paczian, T., Parrello, B., Pusch, G. D., Reich, C., Stevens, R., Vassieva, O., Vonstein, V., Wilke, A., and Zagnitko, O. 2008. The RAST server: Rapid annotations using subsystems technology. BMC Genomics 9:75.
- Bender, C. L., Alarcón-Chaidez, F., and Gross, D. C. 1999. *Pseudomonas syringae* phytotoxins: Mode of action, regulation, and biosynthesis by peptide and polyketide synthetases. Microbiol. Mol. Biol. Rev. 63:266-292.
- Bentley, S. D., Chater, K. F., Cerdeñatarraga, A. M., Challis, G. L., Thomson, N. R., James, K. D., Harris, D. E., Quail, M. A., Kieser, H., Harper, D., Bateman, A., Brown, S., Chandra, G., Chen, C. W., Collins, M., Cronin, A., Fraser, A., Goble, A., Hidalgo, J., Hornsby, T., Howarth, S., Huang, C. H., Kieser, T., Larke, L., Murphy, L., Oliver, K., O'Neil, S., Rabinowitsch, E., Rajandream, M. A., Rutherford, K., Rutter, S., Seeger, K., Saunders, D., Sharp, S., Squares, R., Squares, S., Taylor, K., Warren, T., Wietzorrek, A., Woodward, J., Barrell, B. G., Parkhill, J., and Hopwood, D. A. 2002. Complete genome sequence of the model actinomycete *Streptomyces coelicolor* A3(2). Nature 417:141-147.
- Bérty, J. 2005. Bioactive microbial metabolites. J. Antibiot. (Tokyo) 58:1-26.
- Bertram, R., Schlicht, M., Mahr, K., Nothaft, H., Saier, M. H., Jr., and Titzmeyer, F. 2004. In silico and transcriptional analysis of carbohydrate uptake systems of *Streptomyces coelicolor* A3(2). J. Bacteriol. 186:1362-1373.
- Bignell, D. R. D., Fyans, J. K., and Cheng, Z. 2014. Phytotoxins produced by plant pathogenic *Streptomyces* species. J. Appl. Microbiol. 116:223-235.
- Bignell, D. R. D., Huguettapia, J. C., Joshi, M. V., Pettis, G. S., and Loria, R. 2010a. What does it take to be a plant pathogen: Genomic insights from *Streptomyces* species. Antonie van Leeuwenhoek 98:179-194.
- Bignell, D. R. D., Seipke, R. F., Huguettapia, J. C., Chambers, A. H., Parry, R. J., and Loria, R. 2010b. *Streptomyces scabies* 87-22 contains a coronafacic acid-like biosynthetic cluster that contributes to plant-microbe interactions. Mol. Plant-Microbe Interact. 23:161-175.
- Bordeleau, E., Ghinet, M. G., and Burrus, V. 2012. Diversity of integrating conjugative elements in actinobacteria. Mob. Genet. Elements 2:119-124.
- Bouchek-Mechiche, K., Gardan, L., Normand, P., and Jouan, B. 2000. DNA relatedness among strains of streptomyces pathogenic to potato in france: Description of three new species, *S. europaeiscabiei* sp. nov. and *S. stelliscabiei* sp. nov. associated with common scab, and *S. reticuliscabiei* sp. nov. associated with netted scab. Int. J. Commun. Syst. 50:91-99.
- Braun, S., Gevens, A., Charkowski, A., Allen, C., and Jansky, S. 2017. Potato common scab: A review of the causal pathogens, management practices, varietal resistance screening methods, and host resistance. Am. J. Potato Res. 94:283-296.
- Bukhalid, R. A., Takeuchi, T., Labeda, D., and Loria, R. 2002. Horizontal transfer of the plant virulence gene, nec1, and flanking sequences among genetically distinct *Streptomyces* strains in the Diastatochromogenes cluster. Appl. Environ. Microbiol. 68:738-744.
- Chapleau, M., Guertin, J. F., Farrokhi, A., Lerat, S., Burrus, V., and Beaulieu, C. 2016. Identification of genetic and environmental factors stimulating excision from *Streptomyces scabiei* chromosome of the toxigenic region responsible for pathogenicity. Mol. Plant Pathol. 17:501-509.
- Dees, M., Sletten, A., and Hermansen, A. 2013. Isolation and characterization of *Streptomyces* species from potato common scab lesions in Norway. Plant Pathol. 62:217-225.
- Delcher, A. L., Phillippy, A., Carlton, J., and Salzberg, S. L. 2002. Fast algorithms for large-scale genome alignment and comparison. Nucleic Acids Res. 30:2478-2483.
- Edgar, R. C. 2004a. Muscle: A multiple sequence alignment method with reduced time and space complexity. BMC Bioinformatics 5:113.
- Edgar, R. C. 2004b. MUSCLE: Multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. 32:1792-1797.
- Edwards, U., Rogall, T., Blöcker, H., Emde, M., and Böttger, E. C. 1989. Isolation and direct complete nucleotide determination of entire genes. Characterization of a gene coding for 16s ribosomal RNA. Nucleic Acids Res. 17:7843-7853.
- Eggers, C. H., Gray, C. M., Preisig, A. M., Glenn, D. M., Pereira, J., Ayers, R. W., Alshahrani, M., Acabbo, C., Becker, M. R., Bruenn, K. N., Cheunq, T., Jendras, T. M., Shepley, A. B., and Moeller, J. T. 2016. Phage-mediated horizontal gene transfer of both prophage and heterologous DNA by ϕ bb-1, a bacteriophage of *Borrelia burgdorferi*. Pathog. Dis. 74:ftw107.
- Goris, J., Konstantinidis, K. T., Klappenbach, J. A., Coenye, T., Vandamme, P., and Tiedje, J. M. 2007. DNA-DNA hybridization values and their relationship to whole-genome sequence similarities. Int. J. Syst. Evol. Microbiol. 57:81-91.
- Goyer, C., Faucher, E., and Beaulieu, C. 1996. *Streptomyces caviscabies* sp. nov. from deep-pitted lesions in potatoes in Québec, Canada. Int. J. Syst. Bacteriol. 46:635-639.
- Guo, Y., Zheng, W., Rong, X., and Huang, Y. 2008. A multilocus phylogeny of the *Streptomyces griseus* 16s rRNA gene clade: Use of multilocus sequence analysis for Streptomyces systematics. Int. J. Syst. Evol. Microbiol. 58:149-159.
- Hao, J. J., Meng, Q. X., Yin, J. F., and Kirk, W. W. 2009. Characterization of a new *Streptomyces* strain, DS3024, that causes potato common scab. Plant Dis. 93:1329-1334.
- Healy, F. G., Wach, M., Krasnoff, S. B., Gibson, D. M., and Loria, R. 2000. The txtAB genes of the plant pathogen *Streptomyces acidiscabies* encode a peptide synthetase required for phytotoxin thaxtomin A production and pathogenicity. Mol. Microbiol. 38:794-804.
- Herbst, D. A., Boll, B., Zocher, G., Stehle, T., and Heide, L. 2013. Structural basis of the interaction of Mbth-like proteins, putative regulators of non-ribosomal peptide biosynthesis, with adenylating enzymes. J. Biol. Chem. 288:1991-2003.
- Hodgson, D. A. 2000. Primary metabolism and its control in Streptomycetes: A most unusual group of bacteria. Adv. Microb. Physiol. 42:47-238.
- Huguettapia, J. C., Badger, J. H., Loria, R., and Pettis, G. S. 2011. *Streptomyces turgidiscabies* Car8 contains a modular pathogenicity island that shares virulence genes with other actinobacterial plant pathogens. Plasmid 65:118-124.
- Huguettapia, J. C., Bignell, D. R., and Loria, R. 2014. Characterization of the integration and modular excision of the integrative conjugative element paist in *Streptomyces turgidiscabies* Car8. PLoS One 9:e99345.
- Johnson, E. G., Joshi, M. V., Gibson, D. M., and Loria, R. 2007. Cello-oligosaccharides released from host plants induce pathogenicity in scab-causing *Streptomyces* species. Physiol. Mol. Plant Pathol. 71:18-25.
- Joshi, M., Rong, X., Moll, S., Kers, J., Franco, C., and Loria, R. 2007a. *Streptomyces turgidiscabies* secretes a novel virulence protein, nec1, which facilitates infection. Mol. Plant-Microbe Interact. 20:599-608.
- Joshi, M. V., Bignell, D. R. D., Johnson, E. G., Sparks, J. P., Gibson, D. M., and Loria, R. 2007b. The Arac/Xyls regulator txtR modulates thaxtomin biosynthesis and virulence in *Streptomyces scabies*. Mol. Microbiol. 66:633-642.
- Joshi, M. V., and Loria, R. 2007. *Streptomyces turgidiscabies* possesses a functional cytokinin biosynthetic pathway and produces leafy galls. Mol. Plant-Microbe Interact. 20:751-758.
- Kers, J. A., Cameron, K. D., Joshi, M. V., Bukhalid, R. A., Morello, J. E., Wach, M. J., Gibson, D. M., and Loria, R. 2005. A large, mobile pathogenicity island confers plant pathogenicity on *Streptomyces* species. Mol. Microbiol. 55:1025-1033.
- King, R. R., and Lawrence, C. H. 1994. Isolation and characterization of thaxtomin-type phytotoxins associated with *Streptomyces ipomoeae*. J. Agric. Food Chem. 42:1791-1794.

- King, R. R., Lawrence, C. H., Clark, M. C., and Calhoun, L. A. 1989. Isolation and characterization of phytotoxins associated with *Streptomyces scabies*. J. Chem. Soc. Chem. Commun. 13:849-850.
- Kreuze, J. F., Suomalainen, S., Paulin, L., and Valkonen, J. P. 1999. Phylogenetic analysis of 16S rRNA genes and PCR analysis of the nec1 gene from *Streptomyces* spp. causing common scab, pitted scab, and netted scab in Finland. Phytopathology 89:462.
- Labeda, D. P., Goodfellow, M., Brown, R., Ward, A. C., Lanoot, B., Vannanneyt, M., Swings, J., Kim, S. B., Liu, Z., Chun, J., Tamura, T., Ouchi, A., Kikuchi, H., Nishii, T., Tsuji, K., Yamaguchi, Y., Tase, A., Takahashi, M., Sakane, T., Suzuki, K. I., and Hatano, K. 2012. Phylogenetic study of the species within the family Streptomycetaceae. Antonie van Leeuwenhoek 101:73-104.
- Lambert, D. H., and Loria, R. 1989a. *Streptomyces acidiscabies* sp. nov. Int. J. Syst. Bacteriol. 39:393-396.
- Lambert, D. H., and Loria, R. 1989b. *Streptomyces scabies* sp. nov. nom. rev. Int. J. Syst. Bacteriol. 39:387-392.
- Lawrence, C. H., Clark, M. C., and King, R. R. 1990. Induction of common scab symptoms in aseptically cultured potato tubers by the vivotoxin, thaxtomin. Phytopathology 80:606-608.
- Leclerc, C., Zebré, A., Enault, J., Racine, P. J., and Leblanc, S. 2017. Evaluation of *Streptomyces* common scab toxins diffusion in potato tubers and through the intestinal barrier. Int. J. Curr. Microbiol. Appl. Sci. 6:1662-1676.
- Leiminger, J., Frank, M., Wenk, C., Poschenrieder, G., Kellermann, A., and Schwarzfischer, A. 2013. Distribution and characterization of streptomyces species causing potato common scab in Germany. Plant Pathol. 62:611-623.
- Leiner, R. H., Fry, B. A., Carling, D. E., and Loria, R. 1996. Probable involvement of thaxtomin A in pathogenicity of *Streptomyces scabies* on seedlings. Phytopathology 86:709-713.
- Lerat, S., Simaubeaunoir, A. M., and Beaulieu, C. 2009. Genetic and physiological determinants of *Streptomyces scabies* pathogenicity. Mol. Plant Pathol. 10:579-585.
- Li, L., Stoeckert, C. J., and Roos, D. S. 2003. Orthomcl: Identification of ortholog groups for eukaryotic Genomes. Genome Res. 13:2178-2189.
- Loria, R., Bignell, D. R., Huguet-Tapia, J. C., Joshi, M. V., Johnson, E. G., Seipke, R. F., and Gibson, D. M. 2008. Thaxtomin biosynthesis: The path to plant pathogenicity in the genus *Streptomyces*. Antonie van Leeuwenhoek 94:3-10.
- Loria, R., Bukhalid, R. A., Creath, R. A., Leiner, R. H., Olivier, M., and Steffens, J. C. 1995. Differential production of thaxtomins by pathogenic *Streptomyces* species in vitro. Phytopathology 85:537-541.
- Loria, R., Bukhalid, R. A., Fry, B. A., and King, R. R. 1997. Plant pathogenicity in the genus *Streptomyces*. Plant Dis. 81:836-846.
- Loria, R., Kers, J., and Joshi, M. 2006. Evolution of plant pathogenicity in *Streptomyces*. Annu. Rev. Phytopathol. 44:469-487.
- McClerklin, S. A., Lee, S. G., Harper, C. P., Nwumeh, R., Jez, J. M., and Kunkel, B. N. 2018. Indole-3-acetaldehyde dehydrogenase-dependent auxin synthesis contributes to virulence of *Pseudomonas syringae* strain DC3000. PLoS Pathog 14:e1006811.
- Meier-Kolthoff, J. P., Auch, A. F., Klenk, H. P., and Göker, M. 2013. Genome sequence-based species delimitation with confidence intervals and improved distance functions. BMC Bioinformatics 14:60.
- Miyajima, K., Tanaka, F., Takeuchi, T., and Kuninaga, S. 1998. *Streptomyces turgidiscabies* sp. nov. Int. J. Syst. Bacteriol. 48:495-502.
- Park, D. H., Kim, J. S., Kwon, S. W., Wilson, C., Yu, Y. M., Hur, J. H., and Lim, C. K. 2003. *Streptomyces luridiscabiei* sp. nov. *Streptomyces puniscabiei* sp. nov. and *Streptomyces niveiscabiei* sp. nov. which cause potato common scab disease in Korea. Int. J. Syst. Evol. Microbiol. 53:2049-2054.
- Pertry, I., Václavíková, K., Gemrotová, M., Spíchal, L., Galuszka, P., Depuydt, S., Temmerman, W., Stes, E., Dekeyser, A., Riefler, M., Biondi, S., Novák, O., Schmülling, T., Strnad, M., Tarkowski, P., Holsters, M., and Vereecke, D. 2010. *Rhodococcus fascians* impacts plant development through the dynamic fas-mediated production of a cytokinin mix. Mol. Plant-Microbe Interact. 23:1164-1174.
- Punta, M., Coghill, P. C., Eberhardt, R. Y., Mistry, J., Tate, J., Boursnell, C., Pang, N., Forslund, K., Ceric, G., Clements, J., Heger, A., Holm, L., Sonnhammer, E. L. L., Eddy, S. R., Bateman, A., and Finn, R. D. 2011. The Pfam protein families database. Nucleic Acids Res. D290-D301.
- Richter, M., and Rosselló-Móra, R. 2009. Shifting the genomic gold standard for the prokaryotic species definition. Proc. Natl. Acad. Sci. USA 106:19126-19131.
- Seipke, R. F., and Loria, R. 2008. *Streptomyces scabies* 87-22 possesses a functional tomatinase. J. Bacteriol. 190:7684-7692.
- Shirling, E. B., and Gottlieb, D. 1966. Methods for characterization of *Streptomyces* species. Int. J. Syst. Bacteriol. 16:313-340.
- Song, J., Lee, S. C., Kang, J. W., Baek, H. J., and Suh, J. W. 2004. Phylogenetic analysis of *Streptomyces* spp. isolated from potato scab lesions in Korea on the basis of 16S rRNA gene and 16S-23S rDNA internally transcribed spacer sequences. Int. J. Syst. Evol. Microbiol. 54:203-209.
- Spaepen, S., Vanderleyden, J., and Remans, R. 2007. Indole-3-acetic acid in microbial and microorganism-plant signaling. FEMS Microbiol. Rev. 31:425-448.
- Stamatakis, A. 2014. RaxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30:1312-1313.
- Stegmann, E., Rausch, C., Stockert, S., Burkert, D., and Wohlleben, W. 2006. The small Mbth-like protein encoded by an internal gene of the balhimycin biosynthetic gene cluster is not required for glycopeptide production. FEMS Microbiol. Lett. 262:85-92.
- Wach, M. J., Krasnoff, S. B., Loria, R., and Gibson, D. M. 2007. Effect of carbohydrates on the production of thaxtomin A by *Streptomyces acidiscabies*. Arch. Microbiol. 188:81-88.
- Wang, Y., Tang, H., Debarry, J. D., Tan, X., Li, J., Wang, X., Lee, T., Jin, H., Marler, B., Guo, H., Kissinger, J. C., and Paterson, A. H. 2012. Mcscanx: A toolkit for detection and evolutionary analysis of gene synteny and collinearity. Nucleic Acids Res. 40:e49.
- Wanner, L. A. 2006. A survey of genetic variation in *Streptomyces* isolates causing potato common scab in the United States. Phytopathology 96:1363-1371.
- Zhang, Y., Bignell, D. R., Zuo, R., Fan, Q., Huguet-Tapia, J. C., Ding, Y., and Loria, R. 2016a. Promiscuous pathogenicity islands and phylogeny of pathogenic *Streptomyces* spp. Mol. Plant-Microbe Interact. 29:640-650.
- Zhang, Y., Fan, Q., and Loria, R. 2016b. A re-evaluation of the taxonomy of phytopathogenic genera *Dickeya* and *Pectobacterium* using whole-genome sequencing data. Syst. Appl. Microbiol. 39:252-259.
- Zhang, Y., and Loria, R. 2017. Emergence of novel pathogenic *Streptomyces* species by site-specific accretion and cis-mobilization of pathogenicity islands. Mol. Plant-Microbe Interact. 30:72-82.
- Zhang, Y., and Qiu, S. 2015. Examining phylogenetic relationships of *Erwinia*, and *Pantoea*, species using whole genome sequence data. Antonie van Leeuwenhoek 108:1037-1046.
- Zhang, Y., and Qiu, S. 2016. Phylogenomic analysis of the genus *Ralstonia* based on 686 single-copy genes. Antonie van Leeuwenhoek 109:71-82.
- Zhou, B., Zhang, M. S., and Ma, X. K. 2016. First report of *Streptomyces bottropensis* causing potato common scab in Hebei province, China. Plant Dis. 101.