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Characterization of Potato Scab Pathogens (*Streptomyces* Species) in Korea

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Abstract

Potato scab, an important disease that affects developing tubers, causes a major problem in potato cultivation. The major potato cultivation areas in Korea are located in two Northern provinces, Gangwon and Gyeonggi, and two Southern provinces, Jeju island, and South Jeolla. In these areas, potato scab is widely distributed and has caused severe problem in potato cultivation. Therefore, potato-growing areas were surveyed for identification and distribution of potato scab pathogens from 1996 to 1999. Pathogenic *Streptomyces* strains were isolated from potato scab lesions and six representative *Streptomyces* species were characterized based on their phenotypic and molecular characteristics including, pathogenicity, physiological and morphological properties, analyses of 16S rRNA genes and 16S-23S ITS region, DNA relatedness, production of thaxtomin A, and the presence of *necl* and *ORFmp* gene homologs. Three species were identified as previously described *Streptomyces scabies*, *S. turgidiscabies*, and *S. acidiscabies*, while other three species having distinct phenotypic properties were identified as novel *S. luridiscabiei*, *S. puniscabiei*, and *S. niveiscabiei*.

Introduction

Potato common scab essentially occurs in all potato-growing areas of the world. Although common scab does not affect total yields, significant economic losses result from reduced marketability due to tuber defects. *Streptomyces scabies* was described as first scab pathogen by Thaxter in 1892 and re-proposed by Lambert and Loria (1989a) as the predominant species causing common scab of potatoes. A distinct acid-tolerant species *S. acidiscabies* induces symptoms similar to *S. scabies* (Lambert and Loria, 1989b). *S. turgidiscabies*, which is distinct from both *S. scabies* and *S. acidiscabies* based on their DNA-DNA homology, ribosomal DNA sequences, and morphological characteristics, causes potato scab in Japan (Miyajima et al. 1998). Another two species, *S. europaeiscabiei* and *S.*

stelliscabiei, also cause common scab of potato in France along with *S. reticuliscabiei*, the causal agent of netted scab (Bouček-Mechiche et al. 2000).

In Korea, potato scab disease was first reported to be associated with diseased tubers (Kim and Lim, 1996) and subsequently, identified as *S. scabies*, *S. acidiscabies*, and *S. turgidiscabies* as major causal pathogens of this disease (Kim et al. 1998a, b). In this study, diseased tubers were collected from the major potato cultivation areas that are located in two Northern provinces, Gangwon and Gyeonggi, and two Southern provinces, Jeju island, and South Jeolla and isolated forty pathogenic strains. The symptoms of potato scab in tubers collected from Jeju island were distinct from those typically seen elsewhere in Korea. Isolates derived from these lesions showed distinctly different morphological features. Therefore, phenotypic and phylogenetic properties of forty pathogenic strains were analyzed along with production of thaxtomin A, and presence of *necl* and *ORFtnp* gene homologs and classified them into six distinct clusters. Three clusters contained the strains, which were similar to typical *S. scabies*, *S. acidiscabies*, and *S. turgidiscabies*. However, other three clusters contained *Streptomyces* spp., which were clearly different from those of known scab pathogens, and identified as three novel *S. luridiscabiei*, *S. puniscabiei*, and *S. niveiscabiei*. In this paper, six representative Korean strains of streptomycetes are selected for their characterization.

Taxonomy

Streptomyces scabies has gray smooth spores, borne in spiral spore-chains, resistant to penicillin (10IU^{-1}) and does not grow in 5 and 6% NaCl (Kim et al. 1996). *S. turgidiscabies* has gray smooth spores, borne in rectiflexuous spore-chains, resistant to penicillin (10IU^{-1}) and oleandomycin ($25\mu\text{g}^{-1}$) and does not produce melanin on peptone agar (Kim et al. 1998b). *S. acidiscabies* has white smooth spores, borne in rectiflexuous spore-chains, grows in 5, 6, and 7% NaCl, resistant to oleandomycin ($100\mu\text{g}/\text{ml}$), produces melanin on tyrosine agar, its growth is inhibited below pH 4.5 by thallium acetate ($10\mu\text{g}/\text{ml}$), phenol, and streptomycin (Kim et al. 1998a). *Streptomyces luridiscabiei* has yellow-white smooth spores, borne in monoverticillus flexuous spore chains, produces melanin on tyrosine and peptone agar and utilizes all ISP sugars: L-Arabinose, D-fructose, D-glucose, D-mannitol, raffinose, rhamnose, sucrose, D-xylose, and i-inositol (Park et al. 2003b). The minimum growth pH is 4.5 and sensitive to 5, 6, and 7% (w/v) NaCl, $100\mu\text{g}$ thallium acetate ml^{-1} , 0.1% (w/v) phenol, 25, $100\mu\text{g}$ oleandomycin ml^{-1} , and $20\mu\text{g}$ streptomycin ml^{-1} . *Streptomyces puniscabiei* has pale orange spiny spores, borne in simple rectus flexuous spore chains (Park et al. 2003b). Melanin is produced on tyrosine agar, but not on peptone agar and utilized all ISP sugars: L-Arabinose, D-fructose, D-glucose, D-mannitol, raffinose, rhamnose, sucrose, D-xylose, and i-inositol. The minimum growth pH is 3.5 and sensitive to $10\mu\text{g}$ and $100\mu\text{g}$ thallium acetate ml^{-1} and $20\mu\text{g}$ streptomycin ml^{-1} . *Streptomyces niveiscabiei* has white smooth spores, borne in simple rectus flexuous spore chains, does not produce melanin on tyrosine or peptone agar, but it utilizes all ISP sugars: L-Arabinose, D-fructose, D-glucose,

D-mannitol, raffinose, rhamnose, sucrose, D-xylose, and i-inositol (Park et al. 2003b). The minimum growth pH is 3.5 and sensitive to 5, 6, and 7% (w/v) NaCl, 20, 100 μ g thallium acetate ml⁻¹, 0.5 μ g crystal violet ml⁻¹, 0.1% (w/v) phenol, 25, 100 μ g oleandomycin ml⁻¹, and 20 μ g streptomycin ml⁻¹.

Pathogenicity

All potato scab pathogenic species of *Streptomyces* are known to produce thaxtomin A of the phytotoxin family. In the case of *S. luridiscabiei* and *S. puniscabiei*, these two pathogens do not produce Thaxtomin A in either the media or on the infected potato tissue. This finding indicates that production of thaxtomin A is not necessary for pathogenicity although it has been reported as a characteristic of potato scab pathogens. The *nec1* and *ORFtnp* genes, which are reported to be related to necrosis and the transposase pseudogene, respectively, are found in the potato scab pathogens (Bukhalid et al., 1998; Goyer et al. 1996). However, these *nec1* and *ORFtnp* homologs are absent in *S. acidiscabies*, *S. luridiscabiei*, *S. puniscabiei*, and *S. niveiscabiei* which were isolated from Korea. These observations revealed that the presence or absence of the *nec1* and *ORFtnp* homologs is not linked to the thaxtomin A biosynthesis genes in the Korean strains *S. luridiscabiei* and *S. puniscabiei*. These results also suggested that novel pathogens may have a unique mechanism for pathogenicity, which may have evolved separately from other known scab pathogens.

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