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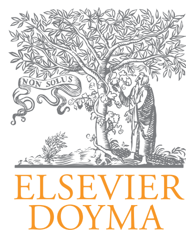

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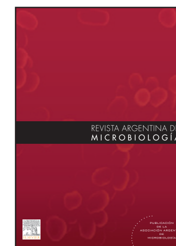

INFORME BREVE

A species-specific method for detecting pathogenic *Streptomyces* species from soil and potato tubers in Argentina

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Abstract
Potato common scab is caused by several soil-inhabiting pathogenic *Streptomyces* species. In the present study, a species-specific PCR method was used to detect *Streptomyces* species in potato tuber lesions and soils. Total genomic DNA from soil samples from six locations and tuber samples from four potato cultivars (Spunta, Shepody, Innovator and Russet Burbank) were assessed. *Streptomyces scabies*, *Streptomyces acidiscabies*, and *Streptomyces turgidiscabies* were detected in soybean, tobacco and potato soils and in all potato varieties except Russet Burbank. The phylogenetic analysis of the sequences obtained confirmed the identification. The method proposed proved to be time-saving and cost effective for the rapid detection of *Streptomyces* species. This is the first report of the detection of *S. acidiscabies* and *S. turgidiscabies* in soils and potato tubers from Argentina.
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PALABRAS CLAVE
Papa;
Sarna;
Streptomyces;
Detección;
S. acidiscabies;
S. turgidiscabies

Método especie-específico para la detección de especies patógenas de *Streptomyces* de suelo y tubérculos de papa en Argentina

Resumen
La sarna común de la papa es causada por varias especies patogénicas de *Streptomyces* habitantes del suelo. En el presente estudio se utilizó un método basado en PCR especie-específico para detectar las especies de *Streptomyces* presentes en lesiones de tubérculos de papa y suelos. Se extrajo ADN genómico total de muestras de suelo de seis localidades y cuatro cultivares de papa (Spunta, Shepody, Innovator y Russet Burbank).

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Streptomyces scabies, *Streptomyces acidiscabies* y *Streptomyces turgidiscabies* fueron detectadas en suelos cultivados con soja, tabaco y papa, y además, en todas las variedades de papa excepto las de Russet Burbank. El análisis filogenético de las secuencias confirmó las identificaciones. Se comprobó que el método propuesto brinda una rápida detección de las especies de *Streptomyces*. Este es el primer informe sobre la detección de *S. acidiscabies* y *S. turgidiscabies* en suelos y tubérculos de papa de Argentina.
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In Argentina, annual potato production is almost 2 million tons[3], and scab disease significantly damages tubers and other root crops. *Streptomyces scabies, Streptomyces acidiscabies,* and *Streptomyces turgisdiscabies* are the best-known causal agents of this disease[12]. *S. scabies* occurs worldwide; *S. acidiscabies* was isolated in the United States, Japan, and Korea, while *S. turgidiscabies* was isolated in the United States, Japan, and Finland[15]. In Argentina, *S. scabies* was identified in 1935 from potatoes with scab symptoms and later isolated from 53 locations in 12 provinces, showing the widespread distribution of the pathogen in soils[9].

The characteristics and pH of the soil can greatly affect the severity of potato scab. Scab is most severe in soils with pH values from 5.2 to 7.0. In some cases, scab control can be achieved by lowering the soil pH. However, "acid scab" is caused by *S. acidiscabies* in soils with pH of 5.0 or below[6]. Thus, measuring the soil pH may be useful to predict the pathogen attack to susceptible potato cultivars if the presence of the pathogen is detected in the soil.

Isolation and identification of *Streptomyces* from potato tubers and soil is time-consuming and laborious owing to the slow growth rate of bacteria and the high diversity of *Streptomyces* species inhabiting scar lesions and soil[5].

The polymerase chain reaction (PCR) assay is less time-consuming and superior to other techniques in its simplicity, rapidity, and sensitivity[12]. Kageyama *et al.*[4] developed a PCR protocol for detection of plant fungal pathogens in the soil. The combination of this method with species-specific primers to amplify the genes of *Streptomyces* spp. could be a useful tool to detect the pathogen in the environment. In the last decade, Tanaka[14] designed species-specific primer combinations to amplify part of the 16S rRNA and Internal Transcribed Spacer (ITS) genes of *S. scabies, S. acidiscabies,* and *S. turgisdiscabies* to detect *Streptomyces* species pathogenic on potato tubers. Thus far, in Argentina *Streptomyces* species have been identified in soils by morphological analysis of the isolates. This study proposes a diagnostic test that does not depend on the isolation, but allows *Streptomyces* species to be detected in soils in a faster and more accurate way, complementing the traditional method. Therefore, the goal of the present research was to detect *Streptomyces* species associated with potato common scab in Argentina using a species-specific PCR method.

Soil samples having different soil textures and crop history were collected from six locations in four provinces of Argentina: 1) Balcarce (37°45'**S**, 58°18'**W**), Buenos Aires province, silty loam soil (Typic Argiudoll), field with long potato crop history; 2) Vedia, (34°29´**S**, 61°32´**W**), Buenos Aires province, sandy loam soil (Entic Hapludoll), soybean crop; 3) Marcos Juárez (32°41'**S**, 62°09'**W**), Córdoba province, loam soil (Typic Argiudoll), soybean crop; 4) Leones (32°67´**S**, 62°30´**W**), Córdoba province, loam soil (Typic Argiudoll), soybean crop; 5) Choele Choel (39°16´**S**, 65°40´**W**), Río Negro province, sandy loam soil (Typic Natrargides), field with recent potato crop history; 6) Juan Bautista Alberdi (27°34´**S**, 65°37´**W**), Tucumán province, loam soil (Typic Argiudoll), tobacco crop. The soil was classified according to the USDA Soil Taxonomy.

The pH values of the soil samples were measured in filtrates of soil (100 g/l) suspended in sterilized distilled water. The soil suspension was shaken in an orbital shaker at 200 r.p.m. (0.5% excentricity), 29 °C ± 0.5 °C for 90 min, and pH measured with a pH meter (SANXIN, Shangai). This operation was repeated three times for each soil sample.

Potato tissue samples were collected from harvested tubers with scab symptoms from Balcarce, a location with more than 110 years of potato crop history. Thirty-one scab lesions were sampled from the following potato cultivars: 1) six lesions from three tubers of the cultivar Spunta; 2) twenty lesions from six tubers of the cultivar Shepody; and 3) five lesions from one tuber of the cultivar Innovator. Potato tubers of the cultivar Russet Burbank from Choele Choel, a location with only two years of potato crop history, were also evaluated. Since these tubers showed no scab lesions, no tissue samples were taken.

Total genomic DNA was extracted from soils according to Kageyama *et al.*[4] as follows: a mixture of 0.2 g soil sample and 0.2 g of sterilized glass beads (1 mm diameter) was suspended in 250 μl extraction buffer: 100 mM Tris-HCl (pH 9.0), 40 mM EDTA, 2% (w/v) sodium dodecyl sulfate (SDS), 0.8% (w/v) skim milk (Difco, USA), and RNase A 200 μg/ml (Nippongene, Japan), vortexed for 1 min. Benzyl chloride (Fluka, Switzerland) (150 μl) was added to the tube, vigorously vortexed for 2 min, and then incubated at 50 °C for 1 h. Following this incubation, 150 μl of 3M NaOAc was added to the suspension, lightly vortexed, and the mixture was incubated on ice for 15 min. This suspension was cleared by centrifugation at 18,000 g for 10 min, and the upper layer was transferred to a clean tube. This step was repeated twice. DNA was precipitated with an equal volume of isopropanol and collected by centrifugation at 18,000 g for 20 min. The resulting pellet was rinsed with 70% ethanol and dried under vacuum. The DNA pellet was dissolved in 200 μl of TE buffer [10 mM Tris-HCl (pH 7.5), 1 mM EDTA]. Three replicates were evaluated from each soil sample. Tissue samples from surface-washed potatoes were cut with a sterilized scalpel and chopped finely, before applying the same protocol as that applied to soil samples. PCR reactions

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