# Serological Detection and Identification of *Streptomyces ipomoea*

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**ABSTRACT**

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Antiserum was produced to *Streptomyces ipomoea*, using unfractionated cellular homogenates as the immunogen. Antibodies to antigens common to *S. ipomoea* and closely related organisms were removed by cross-absorption of the antiserum with extracts from selected strains. Selection of strains was based on antigens detected by western blot analysis, which revealed distinct qualitative differences in antigens shared between *S. ipomoea* and the other strains. Both gel diffusion and ELISA serological tests were used to identify *S. ipomoea* in pure cultures. However, the sensitivity of ELISA was necessary to detect *S. ipomoea* in symptomatic root tissue. The assay was compared with accepted isolation procedures using 114 symptomatic roots collected from 10 fields. *S. ipomoea* was detected in 107 samples by ELISA and in 109 samples by isolation.

*Streptomyces ipomoea* (Person & Martin) Waks. & Henrici causes a disease of sweet potato (*Ipomoea batatas* (L.) Lam.) roots commonly designated "pox" or "soil rot" (1,4,12). This disease is characterized by dark brown to black lesions on the fibrous and fleshy roots. Lesions may be circular or may girdle the root, inhibiting the enlargement of the infected region (4,12). These symptoms are diagnostic for severe infections of this disease. However, injury caused by some insect pests and other stresses of sweet potato may resemble pox symptoms caused by late-season infections.

Confirmed diagnoses of *S. ipomoea* infections are frequently required to document noncompliance with certified seed standards and to provide farmers with reliable information on which to base management decisions. Confirmation of *S. ipomoea* infection currently requires that the organism be isolated in pure culture and tested for pathogenicity. This may require up to 4 wk because of the slow rate of growth of *S. ipomoea* (7,8).

The objective of this investigation was to develop a serological assay for infected sweet potato tissue that could distinguish *S. ipomoea* from closely related organisms that frequently occur in or on sweet potato roots. Preliminary results of this research have been reported (9).

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## MATERIALS AND METHODS

Pathogenic strains of *S. ipomoea* and of contaminating nonpathogenic *Streptomyces* organisms were obtained from naturally infected, fleshy and fibrous sweet potato roots as described previously (8). In addition, type strains of *S. bluensis* and *S. coeruleoroseus*, 10 strains of *Streptomyces* closely related to *S. ipomoea* (A. J. Lyons, NRRL, Peoria, IL), and a strain of *S. scabies*, all of which are nonpathogenic to sweet potato, were used as reference cultures in these experiments. Three or more serial, single-colony transfers were made of each strain before it was stored in sterile water at 12 C. To serve as a source for serological assays and to produce inoculum for pathogenicity assays, each strain was grown on an enriched medium (2) for 5–7 days at 36 C. Pathogenic and nonpathogenic strains were distinguished by the sweet potato root slice assay (7).

Antiserum to *S. ipomoea* (strain NC-5) was produced in New Zealand white rabbits. The immunogen was prepared by suspending colonies from 5- to 7-day-old cultures of *S. ipomoea* in 0.02 M Tris-HCl (pH 7.2), homogenizing in a glass tissue homogenizer, and centrifuging (10,000 *g*) for 10 min. The supernatant was adjusted to $A_{280nm} = 1.4$ and mixed with an equal volume of Freund's complete adjuvant before immunizing the rabbits by intramuscular injections at weekly intervals. Serum was collected beginning 4 wk after the initial injection. Immunizations were continued during the serum collection to maintain antibody titer. Antibodies common to pathogenic and nonpathogenic strains were removed by cross-absorption of the antiserum to NC-5 with a similarly prepared extract from nonpathogenic strains NC-12 or NC-14.

The specificity and sensitivity of the antiserum prepared against *S. ipomoea* was determined by double-diffusion assays, enzyme-linked immunosorbent assay (ELISA), and immunoblots of electrophoretic protein profiles (western transfers) transferred onto nitrocellulose membranes. Double-diffusion medium consisted of 0.5% agarose, 0.1% $NaN_3$, and 0.13 M NaCl. Extracts for double-diffusion assays from strains grown on growth media were prepared in 0.02 M Tris-HCl (pH 7.2) containing 0.15 M NaCl according to the methods used for immunogen preparation. A slightly modified (10) double-antibody sandwich ELISA procedure (3) was used to compare pathogenic strains of *S. ipomoea* with nonpathogenic strains and to assay symptomatic sweet potato root tissue. Extracts of strains grown in pure culture and root tissue were prepared similarly to the immunogen, using the ELISA conjugate buffer as the diluent. Necrotic tissue from one to four root lesions was combined in 1 ml of conjugate buffer for each sample. Coating and conjugated antibodies were used at 2–5 $\mu$g/ml.

Electrophoretic protein profiles from each of the strains were serologically compared after transfer to nitrocellulose membranes. Crude extracts in electrophoresis dissociation buffer were prepared similarly to the immunogen and electrophoresed on 10–20% linear, sodium dodecyl sulfate (SDS) polyacrylamide gels in the Laemmli buffer system (6). Proteins were electrophoretically transferred to nitrocellulose with a BioRad Transblot apparatus (BioRad, Richmond, CA) at 80V for 5 hr and serologically detected by the method of Towbin et al (14). The membrane was incubated in the first antibody (rabbit anti-*S. ipomoea*, 5 $\mu$g/ml) for 16 hr at room temperature, then in the second antibody (horseradish peroxidase-conjugated goat antirabbit, 0.3 $\mu$g/ml) for 1 hr at room temperature followed by incubation in substrate (4-chloro-1-naphthol).

## RESULTS

An antiserum that reacted strongly with pathogenic strains of *S. ipomoea* was produced in rabbits immunized weekly with intramuscular injections of homogenates of *S. ipomoea* cells combined with Freund's complete adjuvant. The preparation was not immunogenic when administered subcutaneously, and it was necessary to continue intramuscular injections as long as serum was collected to maintain the

anti-*S. ipomoea* antibody titer. The specificity of the antiserum for *S. ipomoea* was variable among the three rabbits immunized. The most specific antiserum was selected for these investigations.

Multiple precipitin bands formed between the homogenates of *S. ipomoea* strains and the antiserum in gel-diffusion tests (Figs. 1 and 2). Lines of identity between the strains were apparent for at least three of the bands (Fig. 1). A common precipitin band was also observed between pathogenic *S. ipomoea* strains and some of the nonpathogenic, *Streptomyces*-like organisms that were frequent contaminants in cultures from naturally infected sweet potato roots. The concentration of these antibodies was significantly reduced by cross-absorbing the antiserum with homogenates from the contaminating strains (NC-12 or NC-14) (Fig. 2). The nonpathogenic, *Streptomyces*-like strains were not detected by gel diffusion or ELISA. ELISA values for other *Streptomyces* strains were also negative (Table 1).

The specificity of the antiserum was tested further to determine if the precipitin lines in the gel-diffusion test represented quantitative or qualitative antigenic relationships between the strains and to further evaluate the effectiveness of the cross-absorption. Nitrocellulose membranes containing protein profiles from homogenates of the strains fractionated on 10–20% linear SDS polyacrylamide gels revealed several distinct antigens for the pathogenic strains ranging in approximate relative molecular weight from 22 to 65 kDa when probed with the antiserum before cross-absorption (Fig. 3). The antigens detected in the homologous (Fig. 3, lane B) reaction also were present in varying amounts in homogenates of the other

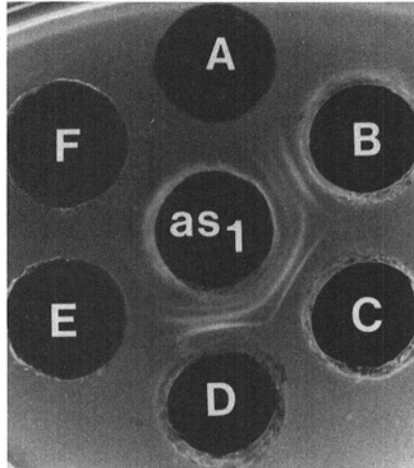


**Fig. 1.** Serological comparison by gel diffusion of pathogenic strains of *Streptomyces ipomoea* (B–D) and other *Streptomyces* spp. (E and F) with antiserum to *S. ipomoea* before ($as_1$) cross-absorption. Each antiserum was incubated with buffer (A) and with *S. ipomoea* strains NC-2 (B), NC-5 (C), NC-85-1 (D) and with two strains of nonpathogenic *Streptomyces* spp.: NC-12 (E) and NRRL B16053 (F).

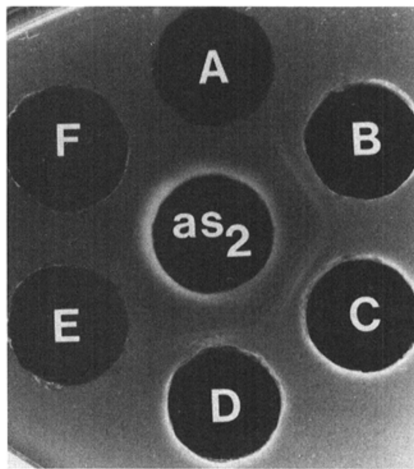


**Fig. 2.** Serological comparison by gel diffusion of pathogenic strains of *Streptomyces ipomoea* (B–D) and other *Streptomyces* spp. (E and F) with antiserum to *S. ipomoea* after ($as_2$) cross-absorption. Each antiserum was incubated with buffer (A) and with *S. ipomoea* strains NC-2 (B), NC-5 (C), NC-85-1 (D) and with two strains of nonpathogenic *Streptomyces* spp.: NC-12 (E) and NRRL B16053 (F).

**Table 1.** Specificity of enzyme-linked immunosorbent assay for *Streptomyces ipomoea* among selected members of strains closely related to *S. ipomoea*

| Culture | Absorbance (405 nm) |
|---|---|
| *S. ipomoea* | |
| NC-1 | 0.457 |
| NC-2 | 0.247 |
| NC-4 | 0.190 |
| NC-5[a] | 0.382 |
| NC-6 | 0.439 |
| *S. bluensis* (type strain) | 0.022 |
| *S. coeruleoroseus* (type strain) | 0.013 |
| *S. scabies* | 0.010 |
| *Streptomyces* spp. | |
| B16050[b] | 0.007 |
| B16051 | 0.017 |
| B16052 | 0.009 |
| B16053 | 0.007 |
| B16054 | 0.009 |
| B16055 | 0.010 |
| B16056 | 0.008 |
| B16057 | 0.011 |
| B16058 | 0.023 |
| B16059 | 0.025 |
| LSD | |
| 0.05 | 0.052 |
| 0.01 | 0.069 |

[a]Strain used as source of immunogen.
[b]NRRL reference number.

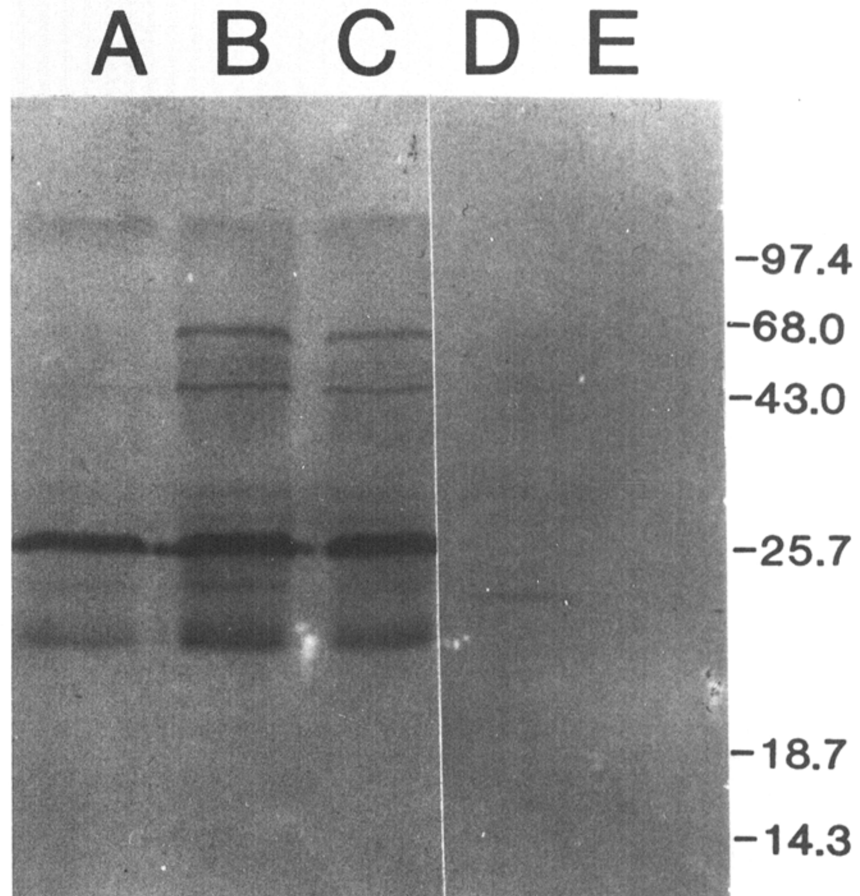


**Fig. 3.** Electrophoretic profiles of antigens extracted from pathogenic strains of *Streptomyces ipomoea* (A = NC-2, B = NC-5, and C = NC-85-1) and other *Streptomyces* spp. (D = NC-12 and E = NRRL B16053) detected by cross-absorbed antiserum to *S. ipomoea*. Molecular weight markers expressed in kilodaltons were phosphorylase b, 97.4; bovine serum albumin, 68; ovalbumin, 43; chymotrypsinogen, 25.7; lactoglobulin, 18.4; and lysozyme, 14.3 (Bethesda Research Labs, Gaithersburg, MD).

**Table 2.** Sensitivity of enzyme-linked immunosorbent assay for *Streptomyces ipomoea* in infected sweet potato root lesions

| Dilution ($-\log_{10}$) | Tissue source Healthy ($A_{405nm}$) | Infected ($A_{405nm}$) |
|---|---|---|
| 1.0 | 0.015 | 0.205 |
| 1.7 | 0.012 | 0.181 |
| 2.0 | 0.009 | 0.150 |
| 2.7 | 0.007 | 0.066 |
| 3.0 | 0.017 | 0.032 |
| 3.7 | 0.015 | 0.022 |
| 4.0 | 0.016 | 0.016 |
| 4.7 | 0.011 | 0.017 |
| 5.0 | 0.017 | 0.024 |
| 6.0 | 0.017 | 0.021 |

pathogenic strains of *S. ipomoea* (Fig. 3, lanes A and C). These antigens also were observed in four other pathogenic strains originating in North Carolina and in four pathogenic strains from Louisiana supplied by C. A. Clark. Many of the nonpathogenic strains did not share any antigens with the pathogenic strains (Table 1, Fig. 3). The reactions of the nonpathogenic strains that did share antigens with the pathogenic strains indicated that only one or two antigens of separate electrophoretic mobilities were detected (Fig. 3).

The specificity of the cross-absorbed antiserum to *S. ipomoea* was further tested by probing homogenates from 13 contaminant strains of *S. ipomoea*-like organisms and 13 closely related strains of *Streptomyces*. Cross-absorption of the antiserum significantly reduced the titer of the antibodies to these antigens to levels barely detectable by the immunoblots (Fig 3, lane D) and to levels lower than the level of sensitivity of ELISA (Table 1).

The ELISA values ($A_{405nm}$) presented in Table 1 are representative of assays of homogenates of pure cultures of the strains of *S. ipomoea*, contaminating strains, and strains known to be closely related to *S. ipomoea*. Only the *S. ipomoea* strains gave $A_{405nm}$ values significantly greater than those of nonreacting controls (Table 1).

ELISA also was used to detect *S. ipomoea* in infected tissue from freshly harvested roots. *S. ipomoea* was detected in dilutions of lesion tissue to $10^{-3}$ (Table 2). Necrotic tissue was routinely combined from two to four lesions for all assays; however, preliminary trials demonstrated that necrotic tissue from a single lesion was sufficient for detection.

The sensitivity of ELISA for *S. ipomoea* was also compared with the standard procedure for isolating *S. ipomoea* from infected tissue onto a minimal medium (8). A total of 114 symptomatic roots from 10 fields were assayed by both methods. *S. ipomoea* was detected in 107 samples by ELISA and in 109 samples by isolating the organism.

Alternative serological methods to ELISA also were evaluated for detection of *S. ipomoea* in sweet potato roots. The microprecipitin test resulted in nonspecific reactions, and the gel-diffusion test lacked the necessary sensitivity. We also were unable to detect antigens in lesion extracts that had been electrophoretically fractionated and transferred to nitrocellulose.

## DISCUSSION

Diagnostic serological assays for plant pathogens other than viruses are being developed with increasing frequency (5,11,13). The multitude of antigens present in these organisms compared with viruses, together with the increased probability that closely related nonpathogenic organisms may occupy the same tissues, has made the development of diagnostic assays for these organisms more complex (13). As is often observed, the specificity of antiserum produced by different rabbits against unfractionated cellular homogenates of *S. ipomoea* was highly variable. The antiserum chosen for these studies recognized the fewest antigens common to both the pathogenic and nonpathogenic strains. The presence of multiple precipitin bands in gel-diffusion assays during preliminary analysis of pure cultures of the different strains indicated that sufficient qualitative differences existed to permit removal of antibodies to common antigens by cross-absorption (Figs. 1 and 2).

Preliminary ELISA revealed quantitative differences among strains that were consistent with the gel-diffusion tests. Western blot comparisons of electrophoretic protein profiles revealed that these quantitative differences measured by ELISA were due to differences in the numbers of antigens recognized by the antiserum in extracts from pathogenic strains and from the contaminating and closely related strains. The western blot analysis also provided the basis for selecting nonpathogenic strains from which extracts were prepared for cross-absorption. Although the antigen(s) detected were consistent within any one strain, there was some variability in the antigens detected among strains. Thus, the strains selected as sources for cross-absorption (NC-12 or NC-14) were representative of the antigens detected in the nonpathogenic strains.

We have demonstrated that the cross-absorbed antiserum can be used in ELISA to identify *S. ipomoea* in pure culture and to detect the organism in infected tissues of commercially grown sweet potato roots and that the detection efficiency was not significantly different from the standard isolation procedure. This antiserum also can be used to identify the antigens specific for *S. ipomoea* (Fig. 3). These antigens could be purified directly from the gels for further study or for use as immunogens for polyclonal or monoclonal antibodies.

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