The latex agglutination test was evaluated for detection of the potato bacterial ring rot pathogen, *Corynebacterium sepedonicum*. The bacterium was detected in infected potato stems and tubers tested and in inoculated eggplant stems. Test reliability was confirmed using blind tests. A minimum of $10^6$ bacterial cells/ml was requisite for observable agglutination. Nonspecific reactions were not observed from healthy extracts of plant tissues. Nonspecific reactions with other bacteria maintained in pure culture were eliminated by addition of bovine serum albumin to sensitized latex preparations.

Introduction

Bacterial ring rot of potato caused by *Corynebacterium sepedonicum* (Spieck. & Kotth.) Skapt. & Burkh. was discovered in North America in 1931 (1). The disease is now found throughout North America and is the major reason for rejection of seed potatoes entered for certification (8). Generally, bacterial ring rot rejections are due to the uniformly strict...
Among all North American potato seed certification agencies and do not reflect high disease incidence within seed lots. Thus, when few or questionable specimens are the basis for diagnosis, considerable debate may ensue.

Recently, a committee of the Pathology Section of the Potato Association of America was established to review available bacterial ring rot diagnostic procedures. Standard criteria for diagnosis were recommended in a report which was endorsed at the 1979 Annual Winter Meeting in Denver, Colorado (7). In all cases, diagnostic criteria included evaluation of vine and/or tuber symptoms. For disputed cases, diagnoses based on symptoms were required to be supplemented by a Gram stain, a serological test, a pathogenicity test, or pathogen isolation and identification.

In general practice, bacterial ring rot diagnosis is based on symptoms and is aided by a confirming Gram stain test. However, the lack of specificity of this test also may be a source of problems (2, 9). Since C. sepedonicum has been shown to be distinct serologically from other Corynebacterium spp. (6), several serological tests have been suggested as specific diagnostic aids to supplement bacterial ring rot determinations (3, 4, 9).

The latex agglutination test has been shown to be a rapid, reliable, sensitive, and simple test for potato viruses S and X in Wisconsin (5). Since this procedure showed promise as a diagnostic assay for C. sepedonicum, this study was initiated to evaluate the application to a bacterial plant pathogen. A preliminary report has been presented (10).

Materials and Methods

Sources of bacterial cultures and production of C. sepedonicum antiserum have been reported (9). The latex agglutination test was performed as described previously (5). Polystyrene spheres (Sigma Chemical Co., St. Louis, MO) now are sold as a 10% rather than a 30% suspension, but dilution-series experiments demonstrated that a 1/15 (v/v) dilution of the 10% suspension in 0.85% NaCl, as with the 30% suspension, produced best results. After preliminary experiments, conjugation of antiserum and latex particles (sensitized latex) was modified by addition of bovine serum albumin (BSA) (Sigma Chemical Co., St. Louis, MO) to the final resuspension buffer.

Test sensitivity was determined by evaluating C. sepedonicum dilution series against antiserum dilutions of sensitized latex. Cell concentrations were adjusted spectrophotometrically with A600nm = 1.0 equivalent to 2 × 10⁹ cells/ml.

Test antigens were prepared either from bacteria maintained in culture on nutrient dextrose agar (NDA: 0.3% beef extract, 1.0% peptone, 1.0% dextrose, and 1.8% agar) or from stem or tuber tissue of plants. From