VERSION: 1.0	**DATE:** 2001
PATHOGEN: Pseudomonas syringae pv. coriandricola	
HOST: coriander/cilantro (Coriandrum sativum)	
COMMON NAME: bacterial blight, leaf spot of coriander	
METHOD: Lcb 7.1 Selective Media Test (STA Laboratories)	
METHOD CLASS: STANDARD (A)	
SAMPLE: 15,000 untreated seeds	

PROCEDURE:

1. Seed sample preparation:

    a. Determine the weight of 100 seeds and calculate weight of required seed, or use seed count information is provided by client.

    b. Add 5,000 seeds (by weight) to each of three autoclaved Erlenmeyer flasks or disposable containers. Add cold, autoclaved extraction buffer (0.85% NaCl) at a ratio of 1:3 (seed weight:mls buffer) plus 1 drop of Tween 20.

2. Extraction of P.s. coriander by Seed Washing:

    a. Shake flasks vigorously on an orbital shaker for approximately 4 hours at ambient temperature.

    b. Filter remaining sample through an autoclaved strainer or autoclaved cheesecloth into an autoclaved beaker. Pour approximately 40-45 ml of filtered seed extract liquid into an autoclaved centrifuge tube.

    c. Transfer several ml's of seed extract liquid to an autoclaved test tube. (This is the direct sample.)

    d. Centrifuge under refrigeration at approximately 10,000 rpm for 10 minutes. Allow time for centrifuge to reach 10,000 rpm.

e. Discard supernatant liquid except for approximately 3-4 ml. Resuspend pellet by vortexing. (This is the concentrated sample.) Alternatively, discard the supernatant and resuspend the pellet in autoclaved dilution buffer (0.85% NaCl).

3. Serial Dilution of Extract:

a. Dilute the direct sample by 1:10 in autoclaved dilution buffer (0.85% NaCl). (This new dilution is designated as d1.)

4. Liquid Plating of P.s. coriander:

a. Pipette approximately 0.1 ml of the d1, direct and concentrated dilution onto each of two labeled plates of KBBC and KBZ using a sterile pipette.

b. Starting with one media type, spread the d1 dilution evenly over the media using a flamed bent rod and turntable, being careful to avoid concentrating the liquid along the edges of the plates. Repeat for the direct then the concentrated dilution.

c. Sterilize rod by flaming and repeat 4.b for the next media type.

d. Incubate plates inverted at 27-30°C for 5-7 days.

5. Evaluation of Semi-Selective Media:

a. Evaluate the plates according to the following schedule, circling suspect colonies. Use a known P.s. coriander culture for comparison

Media	**Evaluate**	**Colony Morphology**
KBBC	5-7 days	Small, flat, translucent with serrated margins, dull blue fluorescence
KBZ	5-7 days	Small, flat, pink, dull blue fluorescence

b. Isolate suspect colonies and a known P.s. coriander culture onto King's B media. After 24-48 hours, compare suspect colonies to positive control.

c. If suspects remain, begin further isolations, biochemical and/or pathogenicity testing as necessary. PSCQR fluoresces dull blue and is oxidase negative on King's B based media.

6. Pathogenicity Testing:

a. Infiltration Method:

1. Prepare a slightly turbid suspension of the suspect by rolling an autoclaved cotton swab over the suspect on the plate and swirling in autoclaved water,

2. Infiltrate the inoculum into the underside of a susceptible coriander leaf (cilantro) using a syringe without the needle.

3. Also inoculate with a known P.s. coriander culture as a positive control and autoclaved water as a negative control.

4. Maintain inoculated plants at 25-32°C for 2-5 days.

5. Symptoms appear as small, irregular water soaked lesions in the infiltration zone. Lesions become black and necrotic. Necrotic spots continue to expand and the leaf blade may collapse and die.

b. Leaf Swab Method:

1. Prepare a slightly turbid suspension of the suspect by rolling an autoclaved cotton swab over the suspect on the plate and swirling in autoclaved distilled water.

2. Swab or spray the inoculum onto the tops and undersides of labeled, susceptible coriander leaves (cilantro). A small amount of carborundum can be used when swabbing to create small leaf wounds.

3. Also inoculate leaves with a known P.s. coriander culture as a positive control and autoclaved water as a negative control.

4. Maintain inoculated plants at 25-32°C for 4-7 days.

5. Symptoms appear as small, dark green, water soaked lesions. Lesions become black and necrotic. Necrotic spots continue to expand and the leaf blade may collapse and die.

MEDIA and BUFFERS:

1. Extraction buffer: 0.85% NaCl, autoclaved

2. Dilution buffer: 0.85% NaCl, autoclaved

3. Semi-selective Media for Isolation:

a. King's B with Boric Acid and Cycloheximide (KBBC) medium

b. King's B with Tetrazolium Chloride (KBZ) medium

4. General Medium for Purification and Growth – King's B medium

REFERENCES:

Taylor, J. D. and Dudley, C. L. 1980. Bacterial disease of coriander. National Vegetable Research Station, Wellesbourne, Warwick CV35 9FF. Plant Pathology. 29:117-121.

Van Buren, Anne M. 1997. Research conducted at STA Laboratories.