A Medium for Cultivation of the B-Strain of *Xanthomonas campestris* pv. *citri*, Cause of Cancrosis B in Argentina and Uruguay

B. I. CANTEROS de ECHENIQUE, Ingeniero Agrónomo, INTA, Bella Vista, Corrientes, Argentina; D. ZAGORY, Assistant Research Scientist, Citrus Research and Education Center, IFAS, University of Florida, Lake Alfred 33850; and R. E. STALL, Professor, Department of Plant Pathology, IFAS, University of Florida, Gainesville 32611

ABSTRACT

Canteros de Echenique, B. I., Zagory, D., and Stall, R. E. 1985. A medium for cultivation of the B-strain of *Xanthomonas campestris* pv. *citri*, cause of cancrosis B in Argentina and Uruguay. Plant Disease 69:122-123.

A culture medium was developed to support the growth of the wild-type B-strain of the citrus canker bacterium. Only rare, fast-growing, probably mutant colonies of the B-strain grew on nutrient agar, King's medium B, Wakimoto's medium, Emerson's medium, and several other media. The wild-type B-strain grew only on a medium containing sucrose (1%), peptone (0.5%), dipotassium phosphate (0.05%), magnesium sulfate (0.03%) and Difco purified agar (1.5%). Agars other than Difco purified agar did not support the growth of B-strain bacteria. This medium facilitates studies of the wild-type B-strain canker organism.

Citrus canker, caused by the bacterium *Xanthomonas campestris* pv. *citri* (Hasse) Dye, poses serious problems to citrus growers in Asia, where it is endemic, as well as in Africa, Oceana, and South America, where it has been introduced. The A-strain, cause of Asiatic or true canker, is the most widespread form of the bacterium and affects most citrus species (1). The B-strain bacterium causes Cancrosis B, or false canker, primarily on lemons in Argentina and Uruguay (4). Although much work has been done concerning the taxonomy, ecology, and control of the A-strain bacterium, little work has dealt with the B-strain, partly because it has been difficult to grow and maintain in culture.

Most cells of the wild-type B-strain citrus canker bacterium show limited growth on nutrient agar (NA) (12); most colonies never grow larger than 0.5 mm in diameter. A few rapidly growing colonies may appear up to 8 or 10 days after transfer to NA, particularly if glucose is added to the medium. On the other hand, the A-strain canker bacterium grows well on NA. To compare the fast- and slow-growing forms of the B-strain bacterium physiologically and pathogenically, we needed a medium on which all cells of the wild-type B-strain bacterium could be cultured routinely. Such work was important because previously published studies concerning the B-strain used Wakimoto's medium and hence selected clones of the rare, fast-growing colony type and not the predominant wild type (4,7–10).

Stall et al (11) failed to culture the B-strain bacterium on NA supplemented with glucose, cellobiose, lemon juice, or lemon rind as well as on Wakimoto's medium or Emerson's medium (6). Therefore, we tested a number of bacterial media to find one suitable for growth of the B-strain bacterium.

MATERIALS AND METHODS

Thirty-two cultures of the canker bacterium were used in the screening of media. Twenty-three A-strain and nine B-strain cultures were collected in Entre Rios and Corrientes provinces, Argentina. B-strain cultures were differentiated from

Table 1. Media tested for the isolation and growth of the B-type citrus canker bacterium

Media components	Concentration (%)
Difco-Bacto agar plus:	1.2
Yeast extract	0.05
Calcium carbonate	1.0
Meat extract	1.0
Soytone	1.0
Lacto-albumin	1.0
Proteose peptone	1.0
Difco-Bacto-peptone	1.0
Dextrin	0.5
Uracil	0.1
Soytone	0.5
Tryptone	0.5
D-Cellobiose	0.5
Hippuric acid	0.1
Amylose	0.5
Alanine	0.1
Norvaline	0.1
Norleucine	0.1
Glucose	0.5
Monopotassium phosphate	0.08
Dipotassium phosphate	0.08
Magnesium sulfate	0.01
Dextrin	0.4
Uracil	0.01
Chlorothalonil (8 mg/ml)	0.25
Kasugamicin (40 mg/ml)	1.0
Difco nutrient broth	0.8
Potato starch	1.0
Difco nutrient broth	0.8
Activated charcoal	0.25
Difco nutrient broth	0.8
or oxoid nutrient broth	0.8
or sucrose	1.0
Difco-Bacto-peptone	0.5
Dipotassium phosphate	0.05
Magnesium sulfate plus:	0.03
Oxoid agar	1.5
Difco-Bacto agar	1.5
Difco purified agar (SMB)	1.5
BBL purified agar	1.5
Noble agar	1.5
Ionagar No. 2	1.5
Agarose	1.5

Florida Agricultural Experiment Stations Journal Series No. 5509.

Accepted for publication 15 July 1984.

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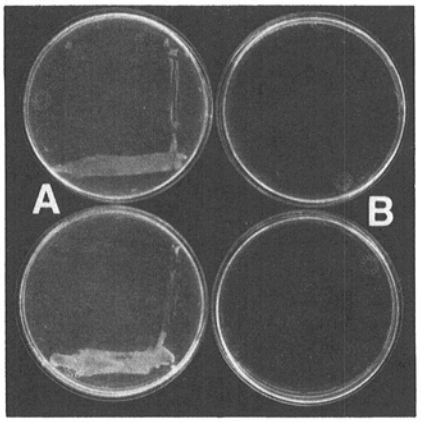

Fig. 1. Two B-strain isolates of the citrus canker bacterium transferred from Difco purified agar medium (SMB) and streaked on **(A)** SMB and **(B)** nutrient agar (NA). After 72 hr of growth, note development of a few small colonies on NA compared with luxuriant growth on SMB.

A-strain cultures by host of origin (B-strain cultures only from lemon) and serologically by M. Messina, INTA, Concordia, Argentina (9). A-strain cultures were isolated from five species of citrus, including lemon. The cultures were maintained in leaves of grapefruit by isolating three lesions from leaves or fruit of naturally infected citrus trees, crushing in 1 ml of sterile water, diluting 1:100, and injecting into young leaves of cultivar Duncan grapefruit in a growth room. After lesions developed, bacteria were plated directly on test media by puncturing each lesion with a sterile needle and immediately stabbing the test medium (3). Bacteria were spread over petri plates with a sterile glass rod. The plates were maintained at 28 C and observed for 2 wk. In all instances, bacteria were stabbed into NA for comparison. The 36 media tested are listed in Table 1.

RESULTS AND DISCUSSION

The B-strain bacteria grew only on sucrose, peptone, salts, and Difco purified agar (SMB) (Table 1). After 48 hr of incubation on SMB, numerous colonies of the B-strain were about 2 mm in diameter. After 5 days on SMB, both A- and B-strains had produced colonies about 5 mm in diameter with abundant slime. The colonies of the B-strain were yellow and appeared similar to A-strain colonies, although the B-strain colonies had a slightly clearer tone. Most colonies of the B-strain on SMB did not grow when transferred to NA (Fig. 1). Colonies of the A-strain grew when transferred to NA. All B-strain isolates grown on SMB were pathogenic after injection-infiltration inoculation into young leaves of Duncan grapefruit. B-strain bacteria could be reisolated from the resulting lesions.

Apparently, most agar formulations contain a factor(s) that prevents the growth of B-strain bacteria. Neither potato starch nor activated charcoal in NA sequestered the presumptive toxic component(s) and B-strain bacteria did not grow. Only Difco purified agar was suitable for growth. SMB medium formulated with 0.25% sucrose (instead of 1%) was also suitable for growth of B-strain bacteria and resulted in less slimy colonies.

The B-strain canker bacterium is not unique among xanthomonads in being difficult to culture on solid media. Hazel et al (5) reported that the addition of agar to two liquid media rendered them unsuitable for growth of *X. fragariae* Kennedy & King. *X. albilineans* (Ashby) Dowson is difficult to isolate and culture, and frequent transfer to fresh medium is necessary to maintain viability (2).

Studies have been initiated to determine the significance of differences between the large-colony and the wild types of B-strain bacteria. Until such studies are completed, we recommend that future work with the B-strain organism from Argentina and Uruguay employ SMB medium to culture the bacterium to ensure that the wild type is represented in the studies.

ACKNOWLEDGMENTS

We thank M. Messina and S. Garran for bacterial isolates and S. Garnsey, J. Graham, D. Roberts, and L. Timmer for helpful criticism of the manuscript. This research was funded in part by a USDA, Tropical Agriculture, Section 406, grant entitled "Epidemiology, control and other hosts of citrus canker."

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