A Selective Medium for and a Definitive Colony Characteristic of Erwinia amylovora

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ABSTRACT

A high sucrose medium (40%) permitted selection of *Erwinia amylovora* from a variety of bacterial species living epiphytically on apple leaf surfaces. The medium had a high plating efficiency; i.e., permitted all *E. amylovora* cells to develop colonies, albeit slowly. Colonies examined at 15 and 30X magnification under oblique light revealed characteristic craters which permitted positive identification of the pathogen. Phytopathology 63:1425-1426

Selective media for species of *Erwinia* (1, 4), and for *Erwinia amylovora* (2, 3), have recently been published. We present here a comparatively simple medium that, in our hands, is highly selective for *E. amylovora* in its natural environment. The use of this medium, when combined with close scrutiny of bacterial colony surfaces, permits identification of the pathogen.

The medium contains 160 g sucrose, 12 g nutrient agar (B B L), 0.8 ml crystal violet (0.1% in absolute alcohol), and 20 ml 0.1% cycloheximide in 380 ml of distilled water. Following autoclaving, the medium was dispensed into gridded plastic petri plates which were dried for 2 hr prior to use or storage. The bacterial suspension to be examined was appropriately diluted and 0.1 ml was spread uniformly over the agar surface with a bent glass rod. After incubation at 28 C for 60 hr, the surface of each bacterial colony was examined at 15 or 30 X magnification with the aid of an obliquely oriented light source.

The selectivity of the medium is illustrated by the following experiment. Leaves of 'Jonathan' apple were collected at random in the field and stored for 5 days under moist conditions in the laboratory to encourage multiplication of saprophytic bacteria on the surfaces. Batches of 10 leaves were then washed in 100 ml of sterile saline for 30 min on a reciprocal shaker. A suspension of *E. amylovora* was added to the washings from each batch of leaves to give a final concentration, determined by plating a saline blank on standard medium, of ca. 2×10^3 cells/ml. After a ten-fold dilution 0.1 ml of washings was plated in triplicate on a standard medium (which contained 20 g sucrose and no crystal violet and was otherwise identical to the selective medium. The numbers of bacterial colonies (*E. amylovora* and saprophytes) detected in a typical 10-leaf sample appear in Table 1.

Only 0.3% of the bacteria plated on the standard

TABLE 1. Number of bacterial colonies isolated/0.01 ml of leaf washings (10 leaves/100 ml saline)

Bacteria	Medium: Standard (5% sucrose)	Medium: Selective (40% sucrose)	Reduction on selective medium (%)
Saprophytes	462[a]	14[a]	97.7
E. amylovora	21[b]	22[a]	0.0

[a] Leaf washings supplemented with *Erwinia amylovora*.
[b] *E. amylovora* plated alone on standard medium at level used to supplement leaf washings.

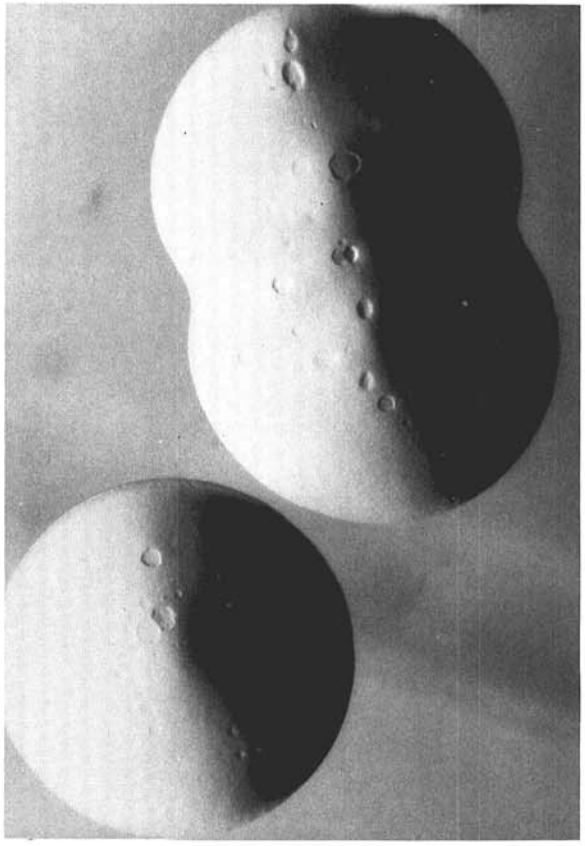

Fig. 1. Cratered surface of colonies of *Erwinia amylovora* viewed with the aid of an oblique light source after 60 hr. Craters may be more numerous in some isolates than shown above.

medium (examined 24 hr after plating) produced colonies on the selective medium. Although the colonies of *E. amylovora* developed more slowly (examined after 60 hr) on the selective medium, their total count was not affected; i.e., the "efficiency of plating" approximated unity.

Of those saprophytes that grew on the selective medium, most colonies were yellow in color when grown on standard medium and were (with few exceptions) sensitive to an *E. herbicola* bacteriophage (EP-35A).

When *E. amylovora* colonies, growing on the selective medium, were viewed at 15 or 30 X magnification with oblique lighting, characteristic craters were observed as noted in Fig. 1. Craters were apparent from 48-72 hr, but were most distinct after 60 hr. More than 50 isolates of *E. amylovora* from a variety of host and geographic origins, virulent and avirulent were examined, and all developed cratered colonies. On the other hand, *E. herbicola* and other saprophytic isolates showed no cratering.

During the past 2 years we have successfully followed population trends of *E. amylovora* on apple leaf surfaces using the selective medium and the characteristic colony cratering habit of the pathogen. With these two criteria it was possible to distinguish *E. amylovora* from saprophytes and other bacteria living epiphytically on apple leaf surfaces. Apparently the pathogen is more competent in surviving the high sucrose environment than most other species commonly found on apple leaves.

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