

SELECTIVE MEDIUM FOR LEUCONOSTOC DETECTION

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The production of slime by microorganisms in the sugar cane juice of sugar mills has been primarily attributed to *Leuconostoc mesenteroides* (McCleskey, Faville, and Barnett, *J. Bacteriol.*, **54**, 697, 1947). In a study of the epiphytic microflora of sugar cane, Mayeux and Colmer (*Sugar J.*, **23**, 28, 1960) reported the presence of many organisms on the growing plant but failed to show the presence of leuconostoc. However, they found leuconostoc present on the harvested cane that was ready for grinding. A study was

In early attempts to make sucrose agar more selective, we used either potassium cyanide or crystal violet alone or in combination with sodium azide, in concentrations from 0.01 to 0.05% of each agent. These were not entirely successful as they either failed to inhibit the undesired organisms or inhibited leuconostoc. A lower concentration of sodium azide used alone was successful. The basal medium was dispensed to give 100-ml amounts, sterilized at 121 C for 15 min, and just prior to use 0.5 ml of

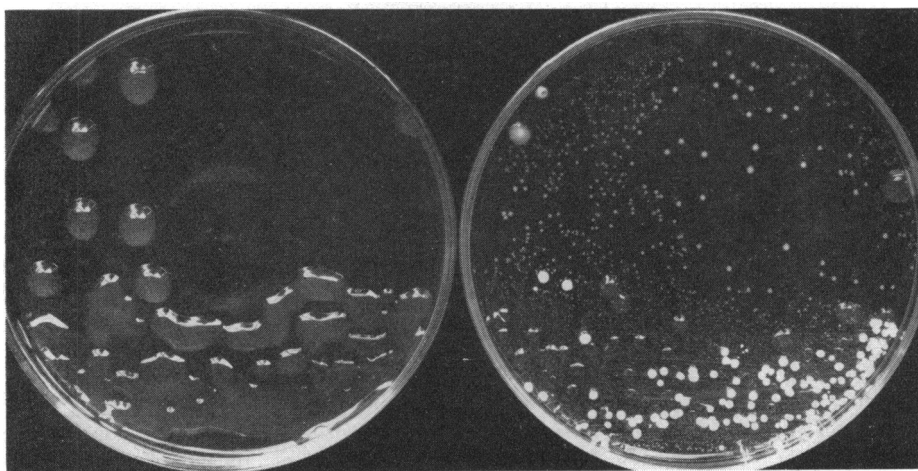


Fig. 1. Ten per cent sucrose agar streaked from a 3-day-old soil enrichment for leuconostoc. Left: plate contains 0.005% sodium azide. Right: control plate.

undertaken to learn if these organisms came from the soil around the cane plant and to correlate the mode of cane harvesting with this possibility.

Preliminary attempts to detect leuconostoc in the soil were disappointing since many slime-forming bacteria grew on the medium of McCleskey et al. (tryptone, 10 g; yeast extract, 5 g; sucrose, 100 g; agar, 20 g; distilled water up to 1,000 ml) and recognition of the leuconostoc-type colony was made difficult. It was, therefore, desirable to develop a medium that would favor the growth of leuconostoc and inhibit the gum-forming bacilli and *Aerobacter aerogenes* commonly encountered.

a 1% sterile sodium azide solution was added to give a final concentration of 0.005%. Comparative yield tests were made with this agar and the regular sucrose agar by smearing 0.1 ml of dilutions of a pure culture of *L. mesenteroides* over the dried surfaces of the two media. The azide agar did not inhibit the growth of the organism and allowed its characteristic colony to appear within 2 days.

Other tests were made with the azide agar in detecting leuconostoc in the soil of cane fields and from the juice obtained from a sugar mill. Fig. 1 illustrates the inhibitory effects of azide agar on the mixed flora present in a sucrose broth enrichment made from soil. Fig. 2 shows

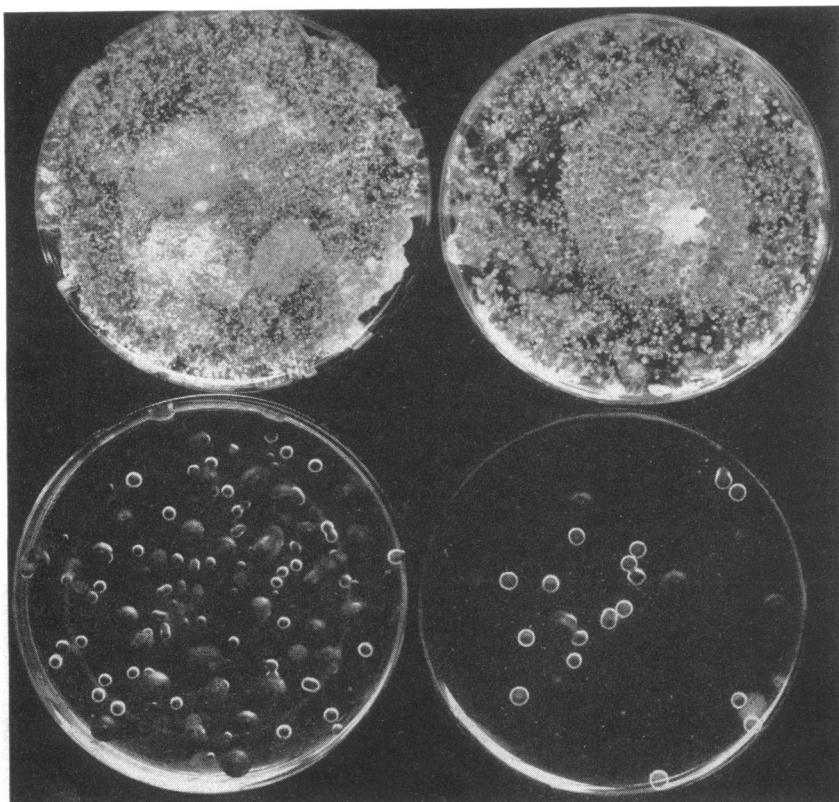


Fig. 2. Ten per cent sucrose agar plates inoculated with dilutions of soil, 1:100 (*top, left*: control; *bottom, left*: contains 0.005% sodium azide) and 1:1,000 (*top, right*: control; *bottom, right*: contains 0.005% sodium azide). Plates are shown inverted.

that the azide agar was equally successful in holding down interfering organisms in dilutions made from such soil without the prior enrichment of leuconostoc. Fig. 3 illustrates the results possible with the selective agar when the cane juice in a mill was examined for leuconostoc in the presence of large numbers of *A. aerogenes* and other organisms. Such plates made with the

azide agar also yielded the four colony types described by McCleskey et al.

Although 0.005% sodium azide had the slight disadvantage of allowing other organisms to appear after 3 to 4 days, concentrations up to 0.03% could be used with a resultant slowing of the appearance of leuconostoc colonies to about 5 days.

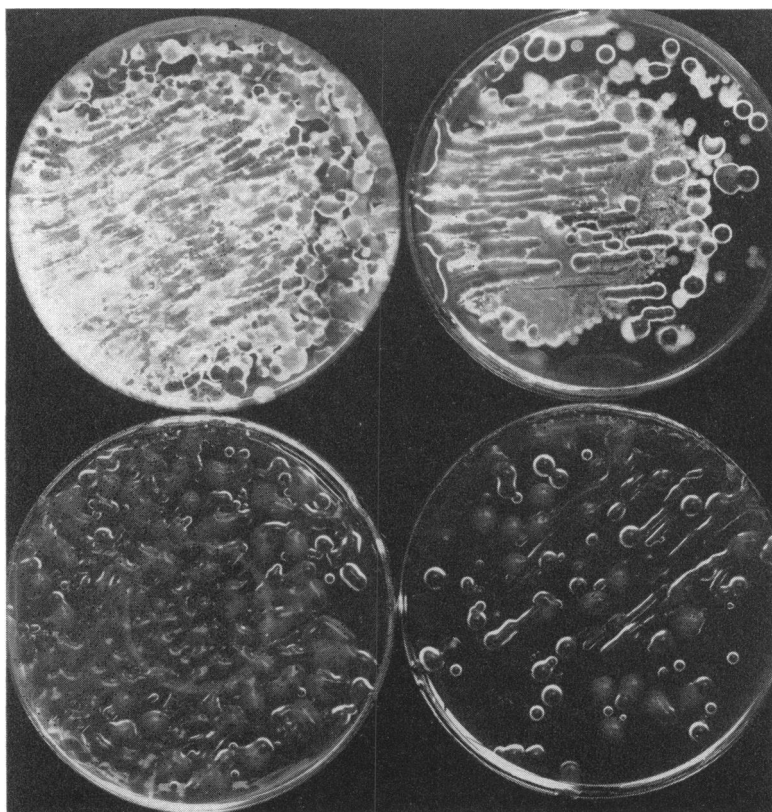


Fig. 3. Ten per cent sucrose agar plates inoculated with dilutions of sugar cane juice, 1:10,000 (*top, left: control; bottom, left: contains 0.005% sodium azide*) and 1:100,000 (*right, top: control; right, bottom: contains 0.005% sodium azide*). Plates are shown inverted.