Detection of Mannitol Formation by Bacteria

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A test is described by means of which formation of mannitol from fructose by lactic acid bacteria can be readily detected. The test is based on removal of interference of residual fructose by dehydration with hydrochloric acid followed by thin-layer chromatography.

The ability of certain bacterial species to convert fructose to mannitol has long been of value in differentiating among the hetero-fermentative lactic bacteria (1, 2), and particularly among lactic acid bacteria responsible for the malo-lactic fermentation in wines (3, 4, 5, 6, 9, 10). Classically, the test involves visual detection of crystal rosettes of mannitol formed when the growth medium is permitted to evaporate (7) over the course of several days or a week.

Although some separation of mannitol from fructose is obtained on thin-layer chromatography with various systems (8), it is not sufficient to permit reliable detection of mannitol formation when residual fructose is present in the growth media. A simple procedure for unequivocally detecting mannitol is described below.

After growth of the bacteria on a medium (peptone, 5 g/liter; tryptone, 20 g/liter; yeast extract, 5 g/liter; fructose, 5 g/liter; Tween-80, 1 ml/liter; pH 5.5; filter sterilized) containing fructose, a sample of broth is mixed with an equal volume of concentrated HCl and heated in a boiling water bath for 15 min. A portion of 10 to 20 μl containing on the order of 50 μg of mannitol and fructose is spotted on suitable thin-layer chromatography plates (plates prepared from a mixture of 1 part Merck Silica Gel G, type 60, 1 part Merck aluminum oxide G, type E, and 3 parts water, followed by drying for 2 to 3 days at room temperature) which are developed with n-butanol-glacial acetic acid-water, 5:1:4. After development, the plates are sprayed with a periodate-benzidine reagent (see reference 8, p. 486) to detect carbohydrates.

Under the conditions of acid concentration and heating described, fructose is dehydrated and fails to produce a spot on the thin-layer chromatography while mannitol is unaffected and yields a spot at low (0.1 to 0.2) Rf. Thus, finding a spot on the thin-layer chromatography indicates production of mannitol from fructose. Omission of the HCl treatment leads to ambiguous results, owing to overlapping spots when residual fructose is present, as is often the case even for cultures capable of converting fructose to mannitol.

This test has been used successfully for detection of the conversion of fructose to mannitol by a number of lactic acid bacteria obtained from Israel wines. The mannitol-positive isolates appeared to be strains of Lactobacillus leichmanii, Lactobacillus plantarum, and Streptobacterium sp., whereas mannitol-negative isolates were identified as Lactobacillus brevis, Leuconostoc oenos, and Leuconostoc dextranicum.

LITERATURE CITED