A Rapid Screening Test for Hydrogen Peroxide Production by Lactobacilli

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A dilution of a broth culture of *Lactobacillus lactis* was evenly spread with a sterile hockey stick on the surface of 15 ml of MRS lactobacilli agar (Difco Laboratories) containing 0.1 ml of peroxidase (0.2 mg/ml) and 0.1 ml of o-tolidine (20 mg/ml). Peroxidase in the presence of the chromogen o-tolidine reacts with hydrogen peroxide to produce a color change in the chromogen. It was assumed that any peroxide metabolically produced by the lactobacilli during colony formation on the agar medium described above would produce similar color changes. The plates were incubated 24 hr at 37 C. Three colony types were selected for isolation from the plates; those with no color zones surrounding them and those with intermediate and large brown zones.

The isolated cultures of lactobacilli were tested for the ability to produce peroxide in refrigerated sterile 10 percent NFMS (Gilliland and Speck, 1975). The cells in 10 ml of MRS broth were harvested by centrifuging in sterile centrifuge tubes at 12,000 x g for 10 min at 2 C. The pellet from each was resuspended in 5 ml of cold sterile 10 percent NFMS and transferred to a 50 ml Erlenmeyer flask containing 20 ml of cold sterile 10 percent NFMS. The flasks containing the samples were incubated for 22 hr at 5 C on a platform shaker to ensure continuous mixing during incubation. Hydrogen peroxide was measured by an enzymatic method described by Gilliland (1969). The number of viable organisms in the test cultures were determined at 0 hr and 22 hr by plating on MRS agar.

More peroxide was produced by the cultures isolated from colonies which produced the larger zones on the "peroxidase agar" than in those with smaller zones. The numbers of viable lactobacilli remained constant over the 22 hr period at 5 C. *L. lactis* cultures do not grow at 5 C and thus do not produce appreciable acid. The peroxide produced by the lactobacilli added to refrigerated foods can inhibit psychrotrophic spoilage organisms (Gilliland and

Speck, 1975). The "peroxidase agar" test described herein could be used as a rapid screening test for selecting cultures for preparing frozen concentrated cultures to be used for such control of psychrotrophic microorganisms in refrigerated food.

Literature Cited

Gilliland, S. E. 1969. Enzymatic determination of residual hydrogen peroxide in milk. J. Dairy Sci. 52:321.

Gilliland, S. E. and M. L. Speck. 1975. Inhibition of psychrotrophic bacteria by lactobacilli and pediococci in nonfermented refrigerated foods. J. Food Sci. 40:903.