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Use of Membrane Filter Technique in the Microbiological Control for the Brewing Industry

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A physical method was developed involving serial filtration with membrane filters for separating yeast cells from bacteria. Such a method eliminates the need for antibiotics previously required to permit differential counting of such populations. All yeast cells filtered were successfully retained and cultivated on a 1.2-μ membrane filter by use of a synthetic medium. All bacteria filtered avoided entrapment on a 1.2-μ membrane filter and were successfully retained and cultivated on a 0.22-μ membrane filter with the same synthetic medium. Final filtrates from these serial filtrations were free from all yeast cells and bacteria when tested with Fluid Thioglycollate Medium.

When viable counts are performed on samples of fermented beers and wines, the technique must include a method that allows the bacterial population to be determined without any interference from yeast cells (1). The present accepted method involves the use of a synthetic medium containing the antibiotic cycloheximide (2, 4). When cycloheximide is present in a concentration of 0.004 g per liter, it effectively suppresses the growth of all brewery culture yeasts, but has no effect upon bacterial growth (3). In this manner, it has been possible for the brewing industry to perform viable plate counts on their products and to determine bacterial population without interference from yeast growth.

The use of membrane filter technique enables the performance of viable counts on these products by a physical separation of yeast cells from the bacteria in the sample to be tested.

MATERIALS AND METHODS

Organisms. Saccharomyces cerevisiae ATCC 2366, *Pediococcus cerevisiae* ATCC 1079, and *Lactobacillus pastorianus* ATCC 8291 were used in this investigation.

Medium. The growth medium used was a double-strength W-L Nutrient Medium without the inclusion of agar. It had the following composition (in grams): tryptone, 10.0; yeast extract, 8.0; dextrose, 100.0; monopotassium phosphate, 1.1; potassium chloride, 0.84; calcium chloride, 0.25; magnesium sulfate, 0.25; ferric chloride, 0.005; manganese sulfate, 0.005; and bromocresol green, 0.044. Distilled water was added qs to make 1,000.0 ml; the *p*H was adjusted with 1 N HCl to 5.5. All components were obtained from Fisher Scientific Co., Pittsburgh, Pa.

Equipment. Millipore Corp. (Bedford, Mass.) Pyrex filter holders XX10-047-00 with RAWP 1.2-μ filter and GSWP 0.22-μ filter were used.

Experimental procedure. Two methods were employed for determining the effective separation of yeast cells from bacteria. In the first method, pure cultures of organisms were filtered to determine the optimal pore size which would retain each organism. The second method was devised to evaluate the separation of yeast cells from bacteria in mixed cultures of the two.

Filter holders were assembled and sterilized as described by Millipore Corp. (Application Data Manual no. 10 and 40, p. 6-7 and p. 13-15, respectively) by use of both the 1.2- and 0.22-μ filters in each of two separate filter holders. Inoculations were made from "second pass" 24-hr broth cultures of *S. cerevisiae*, *P. cerevisiae*, and *L. pastorianus*. Broth cultures were serially diluted by a factor of 10^{-8}, and 10.0 ml of the dilution was aseptically pipetted into 990.0 ml of sterile distilled water. A 100-ml amount of this final dilution was filtered, so that 1.0 ml of the 10^{-8} dilution was inoculated onto each filter. After filtration with the 10^{-8} dilution, each filter was washed with three 100-ml samples of sterile distilled water to free organisms attached to the sides of the filter holder.

Each filtration was first performed through a 1.2-μ filter; the filtrate was then aseptically transferred to a second filter holder and was filtered again through a 0.22-μ filter. This filter was again washed with three 100.0-ml samples of sterile distilled water.

The filters were removed from the filter holders with sterile forceps and were aseptically placed on an absorbent pad saturated with the medium. Filters were superimposed on the absorbent pads in a manner which avoided the formation of air bubbles between the filter and the pad. Petri dishes holding the pads and filters were inverted and incubated for 24 hr at 37.5 C.

Of the final filtrates, 1.0 ml amounts were asepti-

cally pipetted into tubes containing sterile Fluid Thioglycollate Medium (Fisher Scientific Co.) to determine whether any organisms were retained in the final filtrate.

RESULTS AND DISCUSSION

In all tests performed with pure cultures of *S. cerevisiae*, retention of the organism was achieved on a filter whose pore size averaged 1.2μ. Filtrates from these filtrations revealed no evidence of growth either on a filter of 0.22-μ porosity or in Fluid Thioglycollate Medium. In all tests performed with pure cultures of *P. cerevisiae*, the organism was not retained on the 1.2-μ filter, but was however retained on the 0.22-μ filter. Filtrates from these filtrations showed no evidence of growth in Fluid Thioglycollate Medium. Pure cultures of *L. pastorianus* exhibited identical results to those encountered with pure strains of *P. cerevisiae*.

Results on recovery of mixed cultures of the tested strains on filters of various pore sizes show that all cells of *S. cerevisiae* were retained on the 1.2-μ filter, whereas all cells of *P. cerevisiae* and *L. pastorianus* were retained on the 0.22-μ filter. Cells of *S. cerevisiae* showed no growth with the 0.22-μ filter; cells of *P. cerevisiae* and *L. pastorianus* avoided entrapment with the 1.2-μ filter. Filtrates from these filtrations when inoculated into Fluid Thioglycollate Medium showed no evidence of growth.

Good growth was encountered by use of the synthetic medium with all strains of organisms used. Growth observed on the surface of the filters was abundant, and yeast growth could be easily distinguished from bacterial growth.

All yeast cells were retained on the 1.2-μ filter, whereas all bacterial cells passed through the 1.2-μ filter and were retained on the 0.22-μ filter. Final filtrates when inoculated into Fluid Thioglycollate Medium showed no evidence of growth, indicating that all organisms were retained on either the 1.2-μ or 0.22-μ filter.

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