A VARIABLE-TILT FERMENTATION RACK
FOR SCREENING ORGANISMS IN MICROFUGE TUBES

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SUMMARY
We designed a variable-tilt microfuge fermentation rack to screen liquid cultures of wild and mutant yeasts for ethanol production. The rack design allows for the evaluation of up to 40 cultures in the space normally required for three 125 mL Erlenmeyer flasks. Microfuge tubes containing 1.0 mL of inoculated medium were placed in the rack and incubated with shaking. This technique gave reproducible rates of ethanol formation in relation to sugar uptake.

INTRODUCTION
Industrial strain improvement plays a major role in the commercial development of fermentation processes. Liquid culture screens are most frequently based on results with conical Erlenmeyer shake flasks. However, other liquid culture systems could be used. Liquid culture screens require more time and media than agar plate screens, so fewer isolates can be tested. However, liquid cultures have an advantage over plate screens in that they can be designed to mimic production conditions more closely (1, 2). In situations where the improved isolates are rare and the variability or testing error of the screen is high, a series of coarse and finer screens can be used to test large numbers of isolates. The initial coarse screens give low resolution but require the least effort and are intended more to remove low titre isolates than to identify high-titre ones. Plate screening is one such coarse screening procedure (3, 4). However, plate screening is not suitable for ethanol production because this trait depends heavily on the availability of oxygen. We needed to screen a large number of yeast mutants for their abilities to carry out mixed sugar fermentations under various aeration conditions. To do this, we developed a microcentrifuge tube technique to evaluate ethanol production in liquid cultures. The technique employs a variable-tilt rack that enables different aeration rates. Replicate microfuge tubes are inoculated at the start of the experiment and multiple tubes are harvested at each time point. This minimizes handling time and material costs. A high resolution screen such as an Erlenmeyer shake flask screen can follow after detecting improved isolates (3, 4).

MATERIALS AND METHODS
Yeast strains
The xylose fermenting yeasts Candida shehatae ATCC 22984, C. shehatae mutant FPL-702, Pichia stipitis CBS 6054 and P. stipitis mutant FPL-061 and Pachysolen tannophilus NRRL Y-2460 were used to evaluate the microfermentation screening technique.

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Description of microfuge fermentation apparatus

The microfermentation rack is designed to accommodate 40 microfuge vials for screening growth and fermentation (Fig. 1).

This apparatus can be easily mounted on a gyrotary incubator shaker platforms. The rack has a adjustable pivot mounts on each end that enable angle of inclination set points of 0, 15, 30, 45, 60 and 90° from the horizontal (Fig. 1). This tilt plus the rotational speed vary the aeration rate. A plexiglass lid holds the tubes in place during rotation. Standard microfuge tubes comprise the cultivation vessels. Each tube is perforated on one side to admit air.

Fermentation of mixed sugars

The cultures were grown on YEPX agar consisting of (g/L) yeast extract, 10; peptone, 20; xylose, 20; agar, 20. Inocula were prepared by scooping cells from a 2-day old YEPX agar plate into sterile water. The cell inoculum was adjusted to an optical density of 10 at 525 nm. Fermentation broth (0.9 mL), containing (g/L) glucose, 32; and xylose, 32 along with (g/L) yeast nitrogen base (Difco), 1.7; urea, 2.27 and peptone, 6.56 (5), was dispensed to replicate sterile microfuge tubes and each was inoculated with 0.1 mL of the cell suspension. The tubes were shaken at 100 or 200 rpm for 3 to 5 days at 25 or 32°C. Following incubation, the optical density was periodically checked and the tubes were centrifuged to obtain a clear supernatant for analysis of ethanol, sugars and other fermentation products by GC and HPLC. The cell pellets were dried and cell dry weight were determined. All microfermentation estimations were done in triplicate.

High performance liquid chromatography (HPLC)

The sugars and byproducts of fermentation were separated by HPLC (Hewlett Packard series 1050, USA) using an Aminex carbohydrate HPX 87C, column (300 x 7.8 cm) maintained at 85°C (5). Sugars were quantitated with with a Hewlett Packard 1047A refractive index detector. The mobile phase was degassed distilled water at a flow rate of 0.5 mL/min at a pressure of 50 to 55 bar. The filtered clear sample (980 µL) was mixed with 20 µL of sucrose (500 g/L) as internal standard before injection.

Gas chromatography (GC)

Ethanol was separated by GC (Perkin Elmer, Sigma 3B, USA) using a Poropack column with initial and final oven temperature, 180°C, detector temperature, 220°C and injector temperature, 200°C. The carrier gas was helium and the detector gas was hydrogen. A flame ionization detector was used (5).

RESULTS AND DISCUSSION

It is necessary to evaluate xylose fermenting yeast mutants in liquid medium in order to determine their fermentative abilities. Our protocols employ selective platings for initial screens followed by liquid fermentations to identify improved strains (6). Because of the large numbers of strains involved, it is necessary to employ a multi-level screening approach.

Before developing this apparatus, test tube racks were suitably mounted at a 45° angle and used as microfuge racks. However, they were not convenient to operate and did not give reproducible ethanol values. Later, a microfuge rack with a fixed angle of 45° was fabricated. This model delayed fermentation by up to 5 days due to non-uniform mixing and low aeration of the fermentation broth. With the fixed 45° angle rack, it was necessary to agitate the rack at high speed once every day for 2 to 3 min. Because of the high surface tension from the small diameter plastic tubes and the small amount of liquid employed, a higher surface area and a higher agitation rate was required in order to obtain adequate aeration. This was best achieved by mounting the rack horizontally. A lid lined with foam rubber held the tubes firmly in place and prevented them from coming out during rotation. A 18 gauge needle was used to pierce the