Short Communication

EFFECTS OF ULTRASONIC TREATMENT ON YEAST BUDDING

FEDERICO FEDERICI

*Istituto di Microbiologia Agraria e Tecnica, University of Perugia,
I-06100 Perugia, Italy*

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For many years ultrasonic irradiation has been extensively used for the disintegration of microorganisms and the release of intracellular materials in order to obtain biologically active preparations (1–4). Sonic waves (usual frequency 20 kHz) cause cell breakage by means of mechanical effects of cavitation, as recently described by DOULAH (5).

DAKUBU (6), while inactivating *Escherichia coli* cells by ultrasounds, found an increase in cell population during the early stages of treatment. We obtained the same results while mildly sonicating yeast cells in the course of a study on the optimization of conditions for detachment from soil and other natural substrates in ecology surveys (7).

This paper describes preliminary results of studies on yeast cell budding under the influence of sonic treatment.

Six strains from the Yeast Collection of the Istituto di Microbiologia Agraria e Tecnica, University of Perugia, Italy, were studied: *Saccharomyces bayanus* IMAT 838; *Saccharomyces rosei* IMAT 2494; *Debaryomyces castellii* IMAT 3473; *Cryptococcus albidus* var. *albidus* IMAT 4744; *Kloeckera apiculata* IMAT 5991; *Rhodotorula pilimanae* IMAT 6410. These yeasts were chosen because they present only very rare clumps, and mostly, single buds, when grown on malt agar.

The yeast strains were grown on Bacto-Malt Agar (Difco) for 48 hr at 25°. In order to obtain 20–30% of budded cells, malt agar-grown cells were shaken in a rich liquid medium (Bacto-Yeast Extract (Difco) 0.5%; dextrose 2.0%) until that value was reached. The cells were then harvested by filtration (Metricel Membrane GN-6—45 mm—0.8 micron), washed with sterile water and suspended in M/15 phosphate buffer, pH 7.0, and the concentration was adjusted to 3.5×10^6 cells/ml.

The sonication treatment was conducted for as long as 90 sec in the progression 10+20+60 sec. The device used was a 20 kHz generator (150 W MSE Mullard

(1)

(2)

(3)

(4)

(5)

(6)

Figs. 1-6. Cell survival (■), variation of maximum (●) and minimum (▲) cell diameters and budding (*) during ultrasonic treatment of the six yeast strains considered. (All experiments were done in triplicate).

Ultrasonic Disintegrator) and the acoustic power output was *ca.* 35 W/cm². During the treatment, the temperature of the suspension was maintained at 20–22°. Viable counts were carried on directly from the sonicated suspensions immediately after the treatment, while samples of cells, harvested by filtration and resuspended in a 1% formaldehyde solution, were examined for residual buds and cell size.

The results of our experiments have been summarized in Figs. 1–6 where cell survival, variation of minimum and maximum cell diameters and residual bud during sonication are given for each yeast.

The survival curves show an initial increase in cell population during the early stage of treatment. This increase could be ascribed to the anticipated separation of mature buds by means of cavitation effects of sonic waves. In fact, the bud percentage continuously decreased during sonication, this effect probably being due to the separation of buds which had completed cytokinesis but were still in the period preceding natural separation. Besides, the population increase was always accompanied by a constant decrease in the average cell diameters, mostly depending on the size of freshly detached buds.

Furthermore, a certain delay in the commencement of inactivation occurred at the same time for all the strains. It is likely that at least part of this lag was due to the increased amount of liberated buds.

Our data essentially agree with those reported by PRINGLE and MOR (8), even though a comparison is difficult due to different growth conditions and to the fact that those authors placed major emphasis on clump breakage.

Moreover, the reaction of this particular cell to sonic treatment could possibly justify the fermentation rate increase after mild sonication, as pointed out by LEMAO and PRUDHOMME (9) and SRIVASTVA and DE (10). This very interesting possibility is now being investigated in detail.

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