

Effects of Alkylbenzene Sulfonates on Yeasts

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Species of *Hansenula* and *Candida* are resistant to high concentrations of anionic alkylbenzene sulfonates and degrade subinhibitory concentrations of these detergents.

Although one report implicated a black yeast-like fungus, *Phialophora jeanselmei*, in the degradation of alkylbenzene sulfonates (2), the role of yeasts in the metabolism of linear (LAS) and

the first time reports on the degradation of anionic alkylbenzene sulfonates by yeasts.

All media were filter sterilized. Inoculum, adjusted to a final density of 10^4 to 10^5 cells/ml via

TABLE 1. Minimal inhibitory concentration of detergents for yeast growth^a

| Species | Code no. | Source | LAS ^b (mg/liter) | ABS ^c (mg/liter) |
|---------------------------------|----------|-----------------------------|--------------------------------|--------------------------------|
| <i>Candida albicans</i> | 580 | Human (urine) | 25 | 10 |
| <i>C. albicans</i> | 634 | Human (urine) | 25 | 10 |
| <i>C. albicans</i> | 744 | Human (urine) | 25 | 10 |
| <i>C. ingens</i> | CDC1034 | Soap dispenser | 40 | 20 |
| <i>Candida</i> sp. | NS1050 | North Sea (water) | 140 | >400 |
| <i>Candida</i> sp. | E1509 | Florida Everglades (water) | 180 | >400 |
| <i>C. krusei</i> | NS1069 | North Sea (water) | 80 | >400 |
| <i>C. krusei</i> | NS1119 | North Sea (water) | 60 | >400 |
| <i>C. lipolytica</i> | NS1094 | North Sea (water) | 40 | 10 |
| <i>C. tropicalis</i> | NCDC | Clinical | 25 | 20 |
| <i>Cryptococcus albidus</i> | B600 | Clinical | 15 | 10 |
| <i>C. albidus</i> | AA-7004 | Antarctica (water) | 25 | 10 |
| <i>Debaryomyces hansenii</i> | NS1109 | North Sea (water) | 40 | 20 |
| <i>Hansenula angusta</i> | Y-1798 | USDA (spoiled orange juice) | 40 | 10 |
| <i>H. angusta</i> | Y-2214 | USDA (spoiled orange juice) | 40 | 10 |
| <i>H. californica</i> | NS1071 | North Sea (water) | 120 | >400 |
| <i>H. californica</i> | NS1072 | North Sea (water) | 150 | >400 |
| <i>H. petersonii</i> | YB-3807 | USDA (human cadaver) | 50 | 20 |
| <i>H. petersonii</i> | YB-3808 | USDA (human cadaver) | 60 | 20 |
| <i>Rhodotorula rubra</i> | NS1088 | North Sea (water) | 40 | 10 |
| <i>Saccharomyces cerevisiae</i> | E311 | Florida Everglades (water) | 15 | 10 |
| <i>Sporobolomyces roseus</i> | NS1055 | North Sea (water) | 15 | 5 |
| <i>Torulopsis glabrata</i> | 471 | Human (urine) | 70 | 25 |

^a Yeast nitrogen base broth plus 0.5% glucose; 7 days of incubation at 25 C.

^b Linear alkylbenzene sulfonate.

^c Branch-chained alkylbenzene sulfonate.

branch-chained (ABS) alkylbenzene sulfonates has not been definitively established. This note describes the effect of LAS and ABS on the growth and respiration of yeasts grown in Yeast Nitrogen Base (Difco) broth [YNB (8)] and for

hemocytometer cell count and dilution procedures, was prepared from cells grown in 5 ml of the YNB broth containing 0.5% glucose for 24 hr at 25 C on a roller drum (28 rev/min) with a tube angle of 80°. The LAS and ABS (lots 2-3

TABLE 2. Degradation of alkylbenzene sulfonates by yeasts in yeast nitrogen base broth with 0.5% glucose

| Species | Code no. | Branch-chained alkylbenzene sulfonate | | Linear alkylbenzene sulfonate | |
|-----------------------|----------|---------------------------------------|--------------------------------|-------------------------------|--------------------------------|
| | | Initial (mg/liter) | Per cent degraded ^a | Initial (mg/liter) | Per cent degraded ^a |
| | | <i>Hansenula californica</i> | NS1071 | 40 | 54 |
| | | 160 | 0 | 100 | 0 |
| <i>H. californica</i> | NS1072 | 40 | 64 | 50 | 54 |
| | | 160 | 15 | 100 | 0 |
| <i>Candida</i> sp. | NS1050 | 40 | 46 | 50 | 50 |
| | | 160 | 0 | 100 | 10 |
| <i>Candida</i> sp. | E1509 | 40 | 59 | 30 | 49 |
| | | 160 | 11 | 150 | 2 |
| <i>C. krusei</i> | NS1069 | 40 | 60 | 30 | 67 |
| | | 73 | 0 | 70 | 39 |
| <i>C. krusei</i> | NS1119 | 40 | 54 | 30 | 62 |
| | | 73 | 0 | 50 | 56 |

^a Decrease in concentration of methylene blue-detergent complex after 7 days.

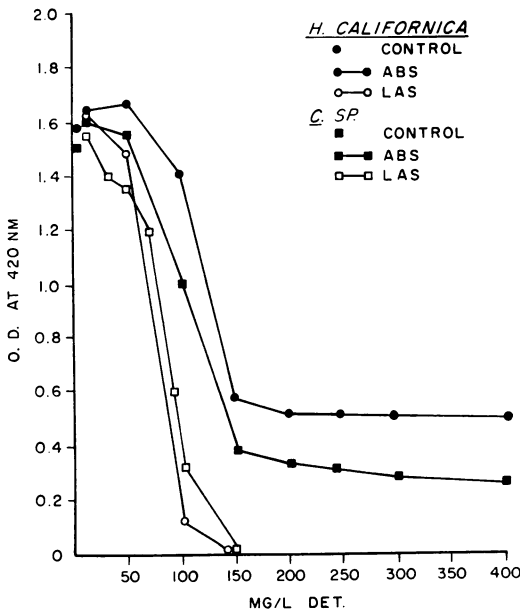


FIG. 1. Effects of increasing concentrations of detergent (DET.) on the growth of *Candida* sp. NS1050 and *Hansenula californica* NS1071 after 4 days at 25 C in yeast nitrogen base broth with 0.5% glucose.

and 5 respectively, Soap and Detergent Association, New York, N.Y.) were employed as chief sources of carbon (5 to 150 mg/liter) in the media. After 7 days of incubation, only negligible growth of the yeasts was evident.

TABLE 3. Effects of linear (LAS) and branch-chained alkylbenzene sulfonate (ABS) on the QO₂ of yeasts

| Detergent | <i>Hansenula californica</i> NS 1071 | | <i>Candida</i> sp. NS 1050 | |
|-------------------|--------------------------------------|-------------------|----------------------------|------|
| | YNB ^a | GYNB ^a | YNB | GYNB |
| None | 25 ^b | 146 | 23 | 100 |
| ABS, 40 mg/liter | 45 | 156 | 29 | 101 |
| ABS, 160 mg/liter | 41 | 133 | 30 | 93 |
| LAS, 50 mg/liter | 36 | 160 | 37 | 90 |
| LAS, 200 mg/liter | 38 | 81 | 34 | 91 |

^a YNB, yeast nitrogen base; GYNB, yeast nitrogen base with 0.5% glucose.

^b Average QO₂ at 25 C (μliters of oxygen per mg of dry weight of cells) of duplicate flasks in 90 min (±4); reaction chambers contained 1 ml of either YNB or GYNB and 1 ml of LAS or ABS; side arm contained 1 ml of washed cell suspension.

Minimal inhibitory concentrations of the LAS and ABS were obtained by adding the detergents in 5.0-mg increments from 5.0 to 30 mg/liter and in 10.0-mg increments from 30 to 400 mg/liter, respectively, to the YNB broth supplemented with 0.5% glucose (Table 1). In general, the yeasts were two to four times more resistant to LAS than to ABS. However, isolates of *Hansenula californica*, *Candida krusei*, and *Candida* sp. (possibly *C. lambica*) were exceptions and grew in the presence of higher concentrations of ABS than of LAS.

Degradation of the detergents by selected yeasts was determined by the methylene blue-complexing method (5) after 7 days of incubation in the yeast nitrogen base broth supplemented with glucose (Table 2). In this procedure, methylene blue is complexed with the intact detergent molecule under acid conditions. The colored complex is extracted with chloroform, and the concentration of detergent is determined spectrophotometrically by comparison with a standard curve. Alterations of the detergent molecules occurred at approximately 50 mg/liter, at which concentrations growth was either stimulated or slightly inhibited (Fig. 1), and the foaming due to the detergents was markedly reduced. Little or no detergent degradation occurred at concentrations which significantly reduced growth.

In the presence of glucose, stimulatory concentrations of the detergents increased oxygen uptake of selected yeasts; however, at growth-inhibitory levels, the oxygen uptake was depressed (Table 3). When glucose was absent, both the growth-stimulatory and inhibitory detergent levels increased oxygen uptake.

The marked resistance of strains of *Candida* and

Hansenula to LAS and ABS and their apparent capacity to metabolize these compounds suggest the use of these yeasts in the removal of alkylbenzene sulfonates from sewage or industrial wastes. Yeasts are of common occurrence in polluted waters and sewage (1, 3) and undoubtedly have been included as part of the inocula in studies on the biodegradation of detergents in sewage (6, 7). The selective effect of high detergent densities may be a factor in explaining the prevalence of *C. krusei* in sewage and urban waters (1, 4).

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