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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 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 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 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 1. *Minimal inhibitory concentration of detergents for yeast growth*[a]

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

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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]
Hansenula californica	NS1071	40	54	50	65
		160	0	100	0
H. californica	NS1072	40	64	50	54
		160	15	100	0
Candida sp.	NS1050	40	46	50	50
		160	0	100	10
Candida sp.	E1509	40	59	30	49
		160	11	150	2
C. krusei	NS1069	40	60	30	67
		73	0	70	39
C. krusei	NS1119	40	54	30	62
		73	0	50	56

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

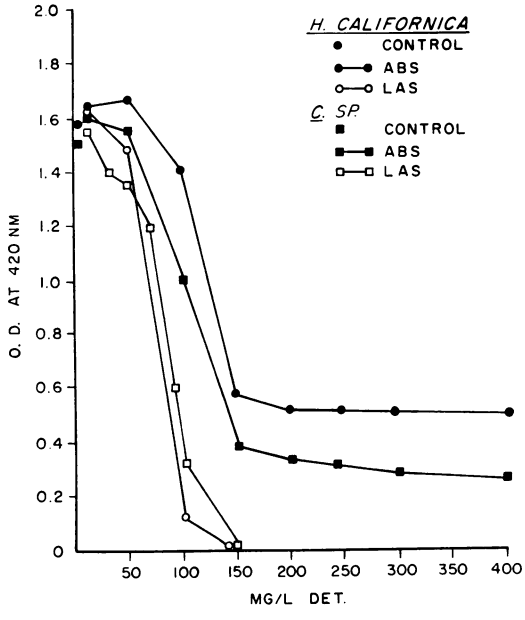

FIG. 1. *Effects of increasing concentrations of detergent (DET.) on the growth of Candida sp. NS1050 and Hansenula californica NS1071 after 4 days at 25 C in yeast nitrogen base broth with 0.5% glucose.*

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 QO_2 of yeasts*

Detergent	*Hansenula californica* NS 1071		*Candida* 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 QO_2 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

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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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LITERATURE CITED

1. Ahearn, D. G., F. J. Roth, Jr., and S. P. Meyers. 1968. Ecology and characterization of yeast from aquatic regions of South Florida. Marine Biology **1**:291–308.
2. Cooke, W. B. 1963. Removal of ABS from solution by common fungus of sewage. Mycopathol. Mycol. Appl. **19**:287–295.
3. Cooke, W. B., and G. S. Matsuura. 1962. A study of yeast populations in a waste stabilization pond system. Protoplasma **57**:163–187.
4. Cooke, W. B., and G. S. Matsuura. 1969. Distribution of fungi in a waste-stabilization pond system. Ecology **50**:689–694.
5. Punghorst, B. A. 1965. Determination of anionic surfactants. Organic Industrial Wastes Characterization Training Manual, U.S. Public Health Service publication 27-3-27-8.
6. Straus, A. E. 1963. Biodegradation of alkyl benzene sulfonates in a simulated septic tank and drain field. Science **142**:244–245.
7. Weaver, P. J. 1965. Testing detergent biodegradability. Soap Chem. Spec. **41**:45–49, 95–96.
8. Wickerham, L. J. 1951. Taxonomy of Yeast. U. S. Dept. Agr. Tech. Bull. **1029**:1–56.

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