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Growth of *Enterobacter cloacae* in the Presence of 25% Sodium Dodecyl Sulfate

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The growth of *Enterobacter cloacae* in 25% sodium dodecyl sulfate is described. The bacteria appeared to tolerate sodium dodecyl sulfate rather than metabolize it. The process was energy dependent, and cell lysis occurred during stationary phase. Extreme detergent resistance may be characteristic of the genus *Enterobacter*.

The interaction between microorganisms and detergents is many sided. Much concern has been directed toward their ability or inability to degrade such detergents as the alkylbenzene sulfonates (7). However, an equally interesting and relatively ignored (10) ecological problem is posed by the capacity of microbes to withstand high detergent concentrations in their environment. The intrinsic sensitivity of proteins, membranes (8), spheroplasts (2), and intact bacterial cells (1, 5, 15) to low detergent concentrations (i.e., ≤1 mg/ml) has been amply documented, and, consequently, we were intrigued to observe microbial growth in a sink containing ca. 1% Alconox. The present communication is devoted to the identification and characterization of that bacterium.

The unknown microbe was streaked onto plates containing sheep blood agar, MacConkey agar, and salmonella-shigella agar (Difco). After incubation at 35°C for 24 h, isolated colonies were recloned and transferred to slants of triple sugar iron agar and lysine iron agar for further testing. These isolates were all oxidase-negative, facultatively anaerobic gram-negative rods which exhibited optimal growth in the pH 6 to 8 range. Accordingly, three isolates were chosen for further characterization via the conventional biochemical tests for the *Enterobacteriaceae* as described by Edwards and Ewing (6) and modified by Brenner et al. (3). Identical results were obtained for the three isolates, and they were identified as *Enterobacter cloacae*.

Early in the characterization, it was found that *E. cloacae* grew abundantly in a defined glucose-salts medium previously used for the fungus *Rhizopus stolonifer* (12). This G medium (composed of 20 g of glucose, 2 g of asparagine, 0.5 g of KH_2PO_4, and 0.28 g of $MgSO_4$ per liter, adjusted to pH 6.8) was the basis for all subsequent tests. The detergent resistance of *E. cloacae* was subsequently characterized with regard to the ability to grow in G medium supplemented with increasing concentrations of sodium dodecyl sulfate (SDS). These SDS-containing media were sterilized by autoclaving; the SDS remained in solution indefinitely at room temperature but quickly precipitated on refrigeration as the temperature dropped below the Krafft point. The growth rates at room temperature were roughly equivalent at all SDS concentrations, but the duration of the lag phase increased as the SDS concentrations present in the inocula and test media became more divergent. These lag periods probably reflect the synthesis of additional proteins in the outer membrane of the *E. cloacae* cells. Localization of these resistance properties in the outer membrane is reasonable since it is well known (9) that gram-negative bacteria are far more resistant to exogenous antimicrobial fatty acids than are gram-positive bacteria.

As indicated in Table 1, *E. cloacae* tolerated SDS at temperatures of up to 40°C. The upper SDS concentration achieved was 25% (wt/vol) at 32°C. These concentrations are, of course, far greater than the critical micelle concentration for SDS. The 25% concentration corresponds to 0.87 M, whereas the critical micelle concentration is in the 1 to 8 mM range, depending on the ambient temperature, ionic strength, etc. This ability to withstand high SDS concentrations remained unaltered when the cells were incubated either anaerobically or in the presence of 0.1% citrate or 0.1% ethylenediaminetetraacetic acid (EDTA) or both. These multicarboxylic acids were added because their chelating capabilities might have introduced synergistic toxicity.

These initial observations raised five further questions concerning the exceptional SDS tolerance exhibited by *E. cloacae*.

(i) Is this tolerance unique to SDS or does it extend to other noxious membrane-active com-

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TABLE 1. *Temperature dependence of SDS tolerance exhibited by E. cloacae*

% SDS	Growth[a] at following temp:			
	22°C	32°C	37°C	40°C
10	++	++	++	++
12.5	++	++	++	++
15	++	++	++	++
17.5	+	++	++	++
20	−	++	++	++
22.5	−	++	++	+
25	−	+	−	−

[a] ++, Over 200 Klett units; +, 100 to 200 Klett units; −, less than 200 Klett units. Identical results were obtained with SDS purchased from Sigma Chemical Co. and BDH Chemical, Ltd.

pounds as well? In this light, we have been able to grow *E. cloacae* at 22°C in G medium supplemented with either 25% (vol/vol) Triton X-100, 6% (vol/vol) ethanol, 2% (wt/vol) urea, 1% (wt/vol) guanidine hydrochloride, 10% (wt/vol) bile salts, 4% (wt/vol) KCl, or 20% (vol/vol) Tween 20, 40, 60, or 80. However, growth in Alconox was restricted to the originally observed 1% (wt/vol) level. Interestingly, Nishikawa et al. (13) recently isolated a bacterium, also tentatively identified as *E. cloacae*, able to grow in 10% of the quaternary ammonium salt benzalkonium chloride.

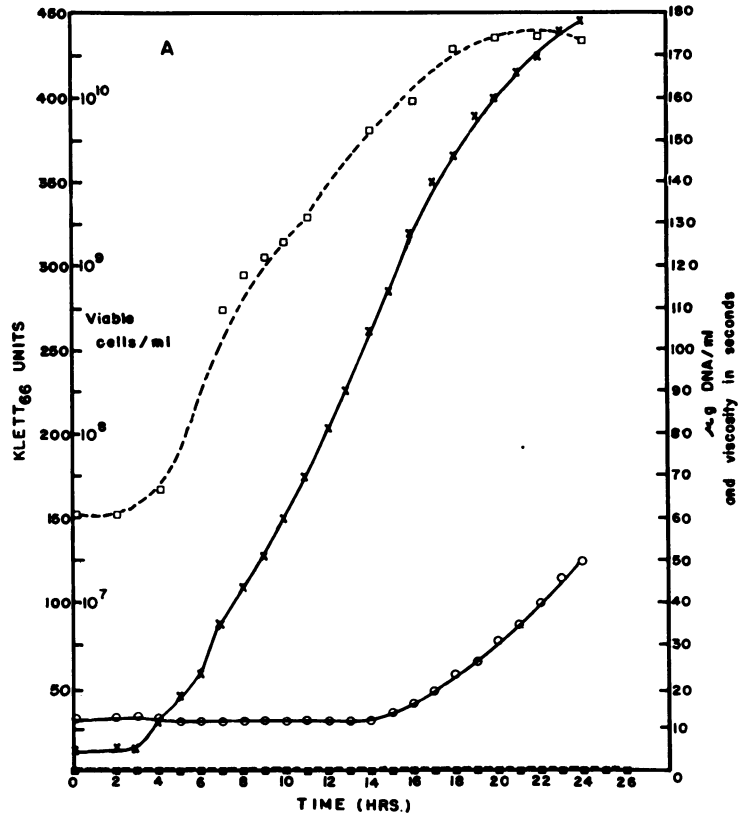
(ii) Our SDS-resistant strain of *E. cloacae* was isolated via a fortuitous detergent-containing selection system. Are other related bacteria equally detergent resistant? Accordingly, four clinical isolates of *Enterobacter* spp. were obtained from the Nebraska State Health Laboratory for comparison. *Enterobacter hafnia*, *Enterobacter aerogenes*, and another strain of *E. cloacae* achieved dense growth in 15% SDS, whereas *Enterobacter agglomerans* barely grew in 1% SDS. Thus, extreme detergent resistance may be characteristic of the genus *Enterobacter* and may be of considerable taxonomic significance. Strains of *E. cloacae* are known to harbor plasmids (11, 16), but we have as yet no evidence whether or not this detergent resistance is plasmid mediated.

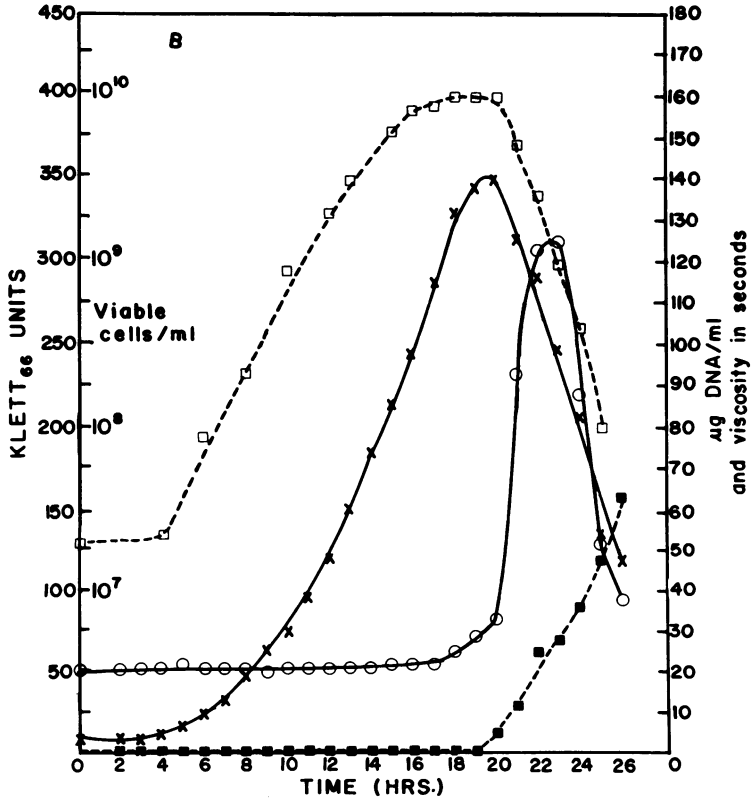
Detergent resistance is a reasonable property to be found in the microbial inhabitants of the bile salt-containing gastrointestinal tract, and it has been exploited frequently by the incorporation of 0.05% deoxycholate in media selective for the enumeration of coliform bacteria. However, extreme detergent resistance, as exemplified by *E. cloacae*, is not a uniform property of all gastrointestinal inhabitants. Cornett and Shockman (5) found that SDS levels of 10 to 20 μg/ml were sufficient to lyse *Streptococcus faecalis*, whereas we have been unable to grow *Escherichia coli*, *Proteus mirabilis*, or *Yersinia enterocolitica* in any medium containing more than 1% SDS.

(iii) Does this remarkable SDS tolerance extend throughout all phases of the bacterium's growth? The ability to grow in SDS does not necessarily imply the ability to remain viable in SDS. Indeed, stationary-phase cultures grown in high SDS concentrations were highly viscous and difficult to clarify by centrifugation; speeds of 14,000 rpm for 30 min were required. These indications of cell lysis were confirmed (Fig. 1B) by demonstrating that the onset of stationary phase was accompanied by increased fluid viscosity, increased deoxyribonucleic acid in the cell-free supernatant (determined by both absorbance at 260 nm and the diphenylamine test [4]), and decreased viable colony-forming units. These postexponential changes were not observed (Fig. 1A) in control cultures lacking SDS. The phenomenon of special detergent sensitivity at the conclusion of active growth was also noted for *P. mirabilis* (15), wherein 0.5% SDS was required to prevent exponential growth but only 0.03% SDS induced lysis of stationary-phase cells.

(iv) Does *E. cloacae* modify the SDS or merely tolerate it? Modification could serve either to detoxify the SDS or to provide the bacterium with a source of carbon, sulfur, and energy. However, even though *Enterobacter* species have been detected (14) among those capable of SDS degradation (at 0.02% concentration), six lines of evidence indicate that our *Enterobacter*-SDS interaction is primarily one of detergent insensitivity. (a) Sufficient SDS remained to lyse cells entering stationary phase (Fig. 1B). (b) The aerobic and anaerobic SDS tolerance levels were indistinguishable. SDS degradation should be an aerobic process (7, 14). (c) The viscosity of the SDS-containing cultures remained constant (Fig. 1B) until just before the

FIG. 1. *Cell lysis during stationary phase of E. cloacae grown in high SDS concentrations. (A) Growth in 0% SDS. (B) Growth in 12% SDS. Symbols: ×, optical density in Klett$_{66}$ units; ■, cell-free deoxyribonucleic acid concentration in micrograms per milliliter as measured by the diphenylamine test (4) with deoxyadenosine as the standard; ○, culture viscosity in seconds (time of fluid transit) determined with a Cannon-Fenske viscosity meter at 22°C; □, viable cells per milliliter. Cells were diluted in 0.002 M phosphate (pH 7.5) and plated onto standard methods agar. Each dilution tube was blended in a Vortex mixer for 30 s. Similar values (±5%) were obtained for stationary-phase cells with the following alternate diluents: (i) 0.15 M NaCl; (ii) 0.01 M KCl, 0.01 M tris(hydroxymethyl)aminomethane (Tris) (pH 7.5), and 0.001 M $MgCl_2$; and (iii) 0.5 M NaCl, 0.02 M sodium citrate, 0.01 M Tris (pH 7.5), and 0.5% SDS.*


A
KLETT66 UNITS
Viable cells/ml
μg DNA/ml and viscosity in seconds
TIME (HRS.)


B
KLETT66 UNITS
Viable cells/ml
μg DNA/ml and viscosity in seconds
TIME (HRS.)

end of exponential growth. If the SDS were being consumed, the viscosity values should have fallen to those characteristic of 0% SDS (Fig. 1A). (d) The generation times calculated from bacterial growth in G medium supplemented with various SDS concentrations were all very similar (1.5 to 2 h). (e) *E. cloacae* could not use purified SDS (four cycles of ethanol precipitation) as its sole source of either carbon or sulfur. Indeed, the final cell yields were somewhat reduced in the presence of SDS. (f) A quantitative assay for SDS was unable to detect any reduction in SDS levels after growth of *E. cloacae* in SDS-containing (5%) media. In this assay, the SDS was precipitated with KOH (K^+ ions raise the Krafft temperature for dodecyl sulfate to 30°C), filtered, dried, and weighed.

(v) Is the SDS tolerance exhibited by *E. cloacae* an energy-requiring process? Apparently it is, since the addition of either sodium azide or dinitrophenol (0.02%) to a mid-exponential culture resulted in complete cell lysis (a drop from 200 to 30 Klett units) within 5 min. Similar additions to cultures without SDS did not result in any decreased turbidity.

In summary, we have demonstrated that *E. cloacae* is capable of exponential growth in 25% SDS. This detergent resistance is probably located in the cell's outer membrane, and an analysis of the outer membrane protein composition in this organism is currently in progress. So far, thin-section transmission electron micrographs of *E. cloacae* grown in 0 and 12% SDS (B. J. Ang and K. W. Nickerson, unpublished data) have failed to detect any morphological consequences of this detergent resistance.

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