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Prevalence of extreme detergent resistance among the *Enterobacteriaceae*

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NOTES

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The detergent-resistance properties of 208 independent isolates of the *Enterobacteriaceae* have been examined. Of these bacterial strains, 200 were able to grow in the presence of ≥5% sodium dodecyl sulfate, including all members of the *Klebsielleae* tribe. This resistance does not appear to be plasmid encoded. It is proposed that detergent-resistant organisms be termed saponotolerant or saponophilic, by analogy with other microorganisms occupying harsh ecological niches. In contrast to their prevalent resistance to anionic detergents, not one of the 208 strains tested was found to grow in the presence of three different cationic detergents. This sensitivity to cationic detergents may be of significance in combating nosocomial infections.

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La résistance à l'action de détergents a été évaluée chez 208 souches non-apparentées d'*Enterobacteriaceae*. Deux cents de ces souches bactériennes incluant tous les membres de la tribu des *Klebsielleae* ont été capables de pousser en présence d'une concentration ≥5% de dodécyl sulfate de sodium. Cette résistance ne semble pas de nature plasmidique. Pour désigner ces organismes résistants aux détergents, il est proposé d'utiliser les termes saponotolérants ou saponophiles par analogie avec d'autres microorganismes qui occupent aussi des niches écologiques hostiles. Contrastant avec la forte prévalence des souches résistantes aux détergents anioniques, on constate qu'aucune des 208 souches n'était capable de pousser en présence de trois détergents cationiques différents. Cette sensibilité aux détergents cationiques pourrait s'avérer intéressante à exploiter dans la lutte aux infections nosocomiales.

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We recently demonstrated (Kramer et al. 1980) that a strain of *Enterobacter cloacae* isolated from a detergent-containing sink was able to grow in the presence of up to 25% (w/v) sodium dodecyl sulfate (SDS). The bacteria appeared to tolerate the SDS rather than metabolize it. Growth was energy dependent and cell lysis commonly occurred during stationary phase. These observations led to the question whether the extreme detergent resistance we observed was an unusual capability resulting from a fortuitous isolation or a common characteristic previously unnoted. To discern the taxonomic distribution of extreme detergent resistance, we decided to concentrate on the *Enterobacteriaceae* because (*i*) gram-negative bacteria are far more resistant to exogenous antimicrobial fatty acids than are gram-positive bacteria (Kabara et al. 1977), and (*ii*) animal gastrointestinal tracts constitute one obvious place for the selection of detergent-resistance capabilities. Accordingly, the 208 independent isolates of the *Enterobacteriaceae* listed in Table 1 were assessed for their capacity to grow in the presence of high concentrations of both cationic and anionic detergents.

These strains were obtained from the Agricultural Research Service Culture Collection, Peoria (19 strains), the University of Nebraska Culture Collection (10 strains), the Centers for Disease Control, Atlanta (98 strains), and at sites on the Back River, Maryland, adjacent to and downstream from the effluent discharge of the Baltimore City sewage treatment facility (81 strains). Each strain was identified by standard procedures (Cowan 1974; Farmer et al. 1980) and none of them had been intentionally stressed by SDS previously. A complete list of the strains employed is available from the second author.

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TABLE 1. Detergent resistance in the *Enterobacteriaceae*

Tribe	Organism	Growth in ≥5% SDS[a]
Escherichieae	*Escherichia coli*	12(16)
	Shigella flexneri	0(1)
	Shigella sonnei	0(1)
Salmonelleae	*Citrobacter freundii*	10(10)
	Salmonella spp.	4(4)
	Salmonella typhi	0(1)
	Salmonella cholerae-suis	1(1)
	Salmonella paratyphi	0(1)
	Salmonella arizonae	1(1)
Klebsielleae	*Klebsiella pneumoniae*	10(10)
	Klebsiella oxytoca	10(10)
	Enterobacter aerogenes	4(4)
	Enterobacter cloacae	70(70)
	Enterobacter agglomerans	14(14)
	Enterobacter gergoviae	25(25)
	Enterobacter sakazakii	35(35)
	Serratia liquefaciens	1(1)
	Serratia marcescens	1(1)
	Hafnia alvei	2(2)

NOTE: The cultures were incubated in nutrient broth (Difco, Detroit) supplemented with 5 or 10% (w/v) SDS (Sigma, St. Louis) for 72 h with rotary agitation (180 rpm) on a G-52 New Brunswick Scientific Co. shaker at room temperature (22–25°C). Growth = ≥100 Klett 66 units; no growth ≤10 Klett 66 units.

[a]Number of isolates exhibiting growth. Total number of isolates tested is given in parenthesis.

Table 1 summarizes our data regarding the ability of 208 isolates from three tribes of the family *Enterobacteriaceae* to grow in the presence of the anionic detergent SDS. As can be seen, SDS resistance is characteristic of this group of bacteria. All strains of the genus *Enterobacter* (158) and of the tribe *Klebsielleae* (182) were SDS resistant. Growth in SDS uniformly achieved final Klett values ≥300. SDS resistance appears to be a fundamental characteristic of the *Klebsielleae* bacteria and not a plasmid-coded acquired characteristic. Plasmid analyses were conducted by the method of Kado and Liu (1981) on 72 *Klebsielleae* strains isolated from the Chesapeake Bay (N. V. Hamlett, unpublished data). They contained from 0 to 8 plasmids, with an average of 2.4 plasmids. Significantly, there were no plasmids common to all isolates and nine SDS-resistant strains contained no detectable plasmids. Some of the *Salmonelleae* and *Escherichiae*, including four strains derived from *Escherichia coli* K12, were also SDS resistant. However, the levels of growth achieved by members of these latter tribes was only in the range of 100–250 Klett units.

None of the 208 strains examined was able to grow in the presence of tetradecyltrimethylammonium bromide (TAB) at either 0.4 or 3.0%. This indication of the sensitivity of the *Enterobacteriaceae* to cationic detergents was confirmed by the further inability of 10 randomly selected strains to grow in the presence of either hexadecylpyridinium chloride or benzalkonium chloride (0.4 and 3.0%), while our original *Enterobacter cloacae* isolate (Kramer et al. 1980) was unable to grow in any of the three cationic detergents at concentrations ranging from 0.1 to 5.0% and pH values ranging from 5.0 to 8.8. The inability of all the bacteria tested to tolerate the cationic detergents was somewhat surprising in view of the report by Nishikawa et al. (1979) of a detergent-resistant bacterium able to grow in 10% benzalkonium chloride. They tentatively identified this bacterium as *Enterobacter cloacae*, but its production of a yellow pigment (Nishikawa et al. 1979) would probably cause it to be reclassified as *Enterobacter sakazakii* (Farmer et al. 1980).

Our data indicate that extreme detergent resistance is a common property in enteric bacteria; 200 of the 208 strains tested were able to grow in the presence of ≥5% SDS, including all members of the *Klebsielleae* tribe. A concentration of 5% SDS is equivalent to 154 m*M*, whereas the critical micelle concentration for SDS is in the 1 to 8 m*M* range, depending on the ambient temperature, ionic strength, etc. SDS resistance was also found in some members of the *Salmonelleae* and *Escherichieae* tribes, and consequently, it is unlikely that extreme detergent resistance will be a useful taxonomic criterion. We have not examined the *Proteeae* and *Yersiniae* tribes, except for the single isolates previously reported to be SDS sensitive (Kramer et al. 1980). The juxtaposition of resistance to anionic detergents and sensitivity to cationic detergents in the *Enterobacteriaceae* may be of significance in combating nosocomial infections.

Thus, there appears to be a class of bacteria characterized by their extreme detergent resistance. We now suggest that all organisms able to grow in the presence of ≥5% SDS, or any other charged detergent, be termed saponotolerant or saponophilic. Similarly, detergent-oversensitive strains, such as the *Escherichia coli* developed especially for biological containment (Schilf and Klingmüller 1981), would be saponophobic. Most of the bacteria listed in Table 1 are probably only saponotolerant because, at least for the one *Enterobacter cloacae* isolate we have studied in detail (Kramer and Nickerson 1984), the presence of 10% SDS decreases the overall cell yield by 20%. Of course, the isolation procedures employed for the cultures studied here would preclude detection of obligate saponophilic bacteria. Our definitions are restricted to charged detergents because we have found (E. J. Ross and V. C. Kramer, unpublished data) that many fungi and gram-positive bacteria were able to grow in the presence of 5% of a neutral detergent such as Triton X-100 but they were completely inhibited by SDS at ≥0.1%.

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COWAN, S. T. 1974. Family I. *Enterobacteriaceae* Rahn 1937. *In* Bergey's manual of determinative bacteriology. 8th ed. *Edited by* R. E. Buchanan and N. E. Gibbons. The Williams & Wilkins Co., Baltimore. pp. 290–340.

FARMER, J. J., III, M. A. ASBURY, F. W. HICKMAN, D. J. BRENNER, and the *Enterobacteriaceae* Study Group. 1980. *Enterobacter sakazakii*: a new species of the "Enterobacteriaceae" isolated from clinical specimens. Int. J. Syst. Bacteriol. **30**: 569–584.

KABARA, J. J., R. VRABLE, and M. S. F. LIE KEN JIE. 1977. Antimicrobial lipids: natural and synthetic fatty acids and monoglycerides. Lipids, **12**: 753–759.

KADO, C. I., and S.-T. LIU. 1981. Rapid procedure for detection and isolation of large and small plasmids. J. Bacteriol. **145**: 1365–1373.

KRAMER, V. C., D. M. CALABRESE, and K. W. NICKERSON. 1980. Growth of *Enterobacter cloacae* in the presence of 25% sodium dodecyl sulfate. Appl. Environ. Microbiol. **40**: 973–976.

KRAMER, V. C., and K. W. NICKERSON. 1984. A transport-dependent energy burden imposed by growth of *Enterobacter cloacae* in the presence of 10% sodium dodecyl sulfate. Can. J. Microbiol. **30**: This issue.

NISHIKAWA, K., S. OI, and T. YAMAMOTO. 1979. A bacterium resistant to benzalkonium chloride. Agric. Biol. Chem. **43**: 2473–2478.

SCHILF, W., and W. KLINGMÜLLER. 1981. Detergent-oversensitive strains of *Escherichia coli* suitable for biological containment. FEMS Microbiol. Lett. **12**: 269–272.