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Association of Increased Recipient Ability for R Factors and Reduced Virulence Among Variants of *Salmonella choleraesuis* var. *kunzendorf*

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During the course of experiments in our laboratory to study the transmission of R factors in weanling pigs, it was frequently observed that, following the introduction of different R factors into strains of *Salmonella choleraesuis* var. *kunzendorf* which were virulent for mice and pigs, hese organisms had altered properties characeristic of rough cultures. They now produced rough colonies, formed granular growth on the bottom of the tube in liquid medium (leaving the broth transparent), no longer reacted serologically with antisera which had been specific for the organisms used to infect, and clumped in the presence of neutral acriflavine. These changes are not unlike those reported to occur in *S. choleraesuis* following in vitro passaging in increasing concentrations of various antibacterials (2); those strains were additionally shown to have a reduced virulence for mice. Recently it was shown that mutants of *S. typhimurium* possess increased recipient ability for R factors (1). One such mutant, Rfer2, was sensitive to coliphages T3, T7, W-31, and P1 and was rough in colonial morphology.

Cultural variants of *S. choleraesuis* var. *kunzendorf* without R factors were isolated from the intestines of pigs which had been infected with a smooth virulent culture. These possessed rough cultural characteristics similar to those observed after introduction of R factors into the smooth virulent parental culture. Furthermore, one of these rough variants was susceptible to coliphages T3 and T7, to which the smooth parental type was insensitive. On the basis of these observations, it seemed possible that R factors were being introduced preferentially into rough cells with reduced virulence.

To test this hypothesis, virulent, smooth paren-

TABLE 1. *Relative competencies of S. choleraesuis var. kunzendorf strain RC221NA, rough variants of RC221NA and E. coli K-12NA as recipients of R factors from E. coli of pig origin*

E. coli donor[b]	Recipient	Selective media[a]	Frequency of R factor transfer[b]
	Expt 1		
P-10	RC221NA	A + NA	0.6×10^{-6}
	29 S.I.-1	A + NA	150.0×10^{-6}
	Expt 2[c]		
(A DS Su)	RC221NA	A + NA	89.0×10^{-6}
	460 S.I.-2	A + NA	48.0×10^{-4}
	K-12NA	A + NA	24.0×10^{-4}
	Expt 3		
105-106-1	RC221NA	DS + NA	3.0×10^{-6}
		SU + NA	24.0×10^{-6}
(DS Su)	29 S.I.-1	DS + NA	41.0×10^{-5}
		SU + NA	11.0×10^{-4}
	26-2(R^-)	DS + NA	86.0×10^{-5}
		SU + NA	19.0×10^{-4}

[a] A = chlortetracycline; DS = dihydrostreptomycin; Su = sulfonamides (sulfaethoxypyridazine was used in these experiments).

[b] The frequencies of transfer of R factors are expressed as the values per introduced donor cell.

[c] In experiment 2, mating mixtures were incubated for 60 min; all other mixtures were incubated for 30 min prior to plating on selective media.

TABLE 2. *Comparison of pathogenicity for mice of S. choleraesuis var. kunzendorf smooth strain RC221NA and rough variants of RC221NA*

Culture	Cultural type[a]	Infective dose[b]	Survivors/total (14 days postinfection)	MST[c]
RC221NA	Smooth	8×10^6	0/50	2.7
		8×10^4	0/49	4.7
		1×10^3	2/9	7.4
		7×10^2	1/10	7.6
		7×10^1	8/10	
26-2(R^+)	Rough	6.5×10^6	10/10	
26-2(R^-)	Rough	8.5×10^6	8/10	
29 S.I.-1	Rough	1.7×10^6	7/10	
		1.7×10^4	9/10	
29 S.I.-1(R^+)	Rough	1.7×10^6	8/10	
		1.7×10^4	9/10	
		1.7×10^2	9/10	
		1.7×10^1	10/10	

[a] All rough cultures were rough in colonial morphology, formed granular growth in broth medium, no longer reacted serologically with antisera specific for parental smooth culture RC221NA, and clumped in the presence of neutral acriflavine.

[b] Number of viable cells per mouse determined by counting colony-forming units on MacConkey agar plates of appropriately diluted samples of the initial 5-hr culture.

[c] Mean survival time (days) of mice that died through 14 days postinfection.

tal culture RC221NA possessing a nontransferable nalidixic acid (NA) marker was compared with rough *S. choleraesuis* var. *kunzendorf* variants 29 S.I.-1, 460 S.I.-2, and 26-2 (R^-), and an NA-marked culture of *Escherichia coli* K-12, designated K-12NA for ability to receive R factors from *E. coli* of pig origin (Table 1). Strains 29 S.I.-1 and 460 S.I.-2 are variants isolated from the small intestines of pigs infected with strain RC221NA. Introduction of an R factor with resistance determinants for chlortetracycline, dihydrosteptomycin, and sulfonamides from *E. coli* of pig origin into strain RC221NA provided rough strain 26-2 (R^+). Strain 26-2 (R^-) is a spontaneous sensitive segregant of strain 26-2 (R^+), which lost all resistance markers associated with the R factor but retained its roughness. As shown in Table 1, all rough cultures were about 100-fold more competent as recipients of R factors from two *E. coli* donors than smooth strain RC221NA. In fact, *Salmonella* variant 460 S.I.-2 was as good a recipient as was *E. coli* K-12NA. Recent experiments indicate that not only do rough cultures serve as better recipients but they are also more competent than smooth cultures when used as donors of R factors. Rough cultures with or without R factors exhibited reduced virulence when injected intraperitoneally into albino Swiss Webster female mice compared to smooth virulent parental cultures RC221 and its derivative RC221NA (Table 2). Mice were maintained continuously on antibiotic-free commercial mouse feed.

We have shown that rough cultures with associated reduced virulence are better recipients of R factors than are their smooth virulent parental counterparts. It is suggested that this roughness may create conditions more favorable to the formation of mating pairs. Studies are now being conducted on surface structures of these more competent cells. We are currently evaluating their increased susceptibility to host-defense mechanisms, such as phagocytosis (3), and whether ability to receive R factors as well as other episomes is directly related to the degree of roughness in this and other genera.

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