JOURNAL OF BACTERIOLOGY, Jan. 1991, p. 55–58
0021-9193/91/010055-04$02.00/0

Vol. 173, No. 1

Expression of the Cloned *Escherichia coli* O9 *rfb* Gene in Various Mutant Strains of *Salmonella typhimurium*

TSUYOSHI SUGIYAMA, NOBUO KIDO,* TAKAYUKI KOMATSU,† MICHIO OHTA, AND NOBUO KATO

Department of Bacteriology, Nagoya University School of Medicine, Showa-ku, Nagoya, Aichi 466, Japan

Received 11 June 1990/Accepted 7 October 1990

To investigate the effect of chromosomal mutation on the synthesis of *rfe*-dependent *Escherichia coli* O9 lipopolysaccharide (LPS), the cloned *E. coli* O9 *rfb* gene was introduced into *Salmonella typhimurium* strains defective in various genes involved in the synthesis of LPS. When *E. coli* O9 *rfb* was introduced into *S. typhimurium* strains possessing defects in *rfb* or *rfc*, they synthesized *E. coli* O9 LPS on their cell surfaces. The *rfe*-defective mutant of *S. typhimurium* synthesized only very small amounts of *E. coli* O9 LPS after the introduction of *E. coli* O9 *rfb*. These results confirmed the widely accepted idea that the biosynthesis of *E. coli* O9-specific polysaccharide does not require *rfc* but requires *rfe*. By using an *rfbT* mutant of the *E. coli* O9 *rfb* gene, the mechanism of transfer of the synthesized *E. coli* O9-specific polysaccharide from antigen carrier lipid to the R-core of *S. typhimurium* was investigated. The *rfbT* mutant of the *E. coli* O9 *rfb* gene failed to direct the synthesis of *E. coli* O9 LPS in the *rfc* mutant strain of *S. typhimurium*, in which *rfaL* and *rfbT* functions are intact, but directed the synthesis of the precursor. Because the intact *E. coli* O9 *rfb* gene directed the synthesis of *E. coli* O9 LPS in the same strain, it was suggested that the *rfaL* product of *S. typhimurium* and *rfbT* product of *E. coli* O9 cooperate to synthesize *E. coli* O9 LPS in *S. typhimurium*.

Lipopolysaccharide (LPS) is composed of three portions bound covalently; lipid A, R-core oligosaccharide, and the O-specific polysaccharide. Genetic and biosynthetic analyses of the lipid A part of LPS have been started, and in *Escherichia coli*, *pgsB* (14) is considered to play an important role in the synthetic pathway of lipid A (for review, see reference 15). By contrast, the genetic and biosynthetic mechanisms of the polysaccharide portion of LPS, R-core oligosaccharide, and O-specific polysaccharide are relatively well known. The genes *kdsA* and *kdsB* (17, 18) in *Salmonella typhimurium* were found to participate in the synthesis of the inner region of R-core. The synthesis of the outer region of R-core is directed by the *rfa* cluster and some housekeeping genes such as *galE*, *galU*, and *pgi* in *S. typhimurium* (11). Both *rfb* and *rfc* are required for O-specific polysaccharide synthesis in *S. typhimurium*, and both *rfb* and *rfe* are required for that in *E. coli* O8 and O9 (6, 16). The biosynthetic mechanism of *rfc*-dependent O-specific polysaccharide synthesis in *S. typhimurium* is widely accepted (6, 16). However, the mechanism underlying *rfe*-dependent synthesis of O-specific polysaccharide in *E. coli* O8 or O9 has not yet been clarified. Exchange experiments of *rfe* between *E. coli* strains or between *E. coli* and *S. typhimurium* revealed that *rfe* was involved in the synthesis of the O-specific polysaccharide in *E. coli* O8 and O9 (20).

Recently we cloned the *rfb* gene cluster of *E. coli* O9 into the pACYC184 cloning vector and characterized some functions of genes in this *rfb* gene cluster. In the present study, we introduced the *E. coli* O9 *rfb* gene into *S. typhimurium* strains possessing defects in various chromosomal genes involved in the synthesis of LPS to investigate the effects of these *S. typhimurium* genes on the synthesis of *E. coli* O9 LPS. *E. coli* O9 *rfb* with the *rfbT* mutation (9) was also introduced into *S. typhimurium* strains to determine the cooperative function of the gene products of *E. coli* O9 *rfbT* and *S. typhimurium rfaL*.

MATERIALS AND METHODS

Strains and media. The bacterial strains used in this study are listed in Table 1. All *S. typhimurium* strains except LB5010 were a gift from P. H. Mäkelä (National Public Health Institute, Helsinki, Finland). *S. typhimurium* LB5010 was a gift from K. Kutsukake (Department of Biology, Faculty of Science, The University of Tokyo, Tokyo, Japan). Media used for the culture of bacteria were the same as those reported previously (7).

Transformation and transduction. *E. coli* O9 *rfb* was recloned into pACYC184 from R'-6 plasmid carrying the *his-rfb* region of *E. coli* O9 (7) and termed pNKB26 (9). Plasmid DNA was amplified in *S. typhimurium* LB5010 to escape restriction by *S. typhimurium*. Extraction of plasmid DNA was carried out by alkaline lysis (12) or the method reported by Sasakawa et al. (19). Plasmid DNA was introduced into various *S. typhimurium* mutants by transformation (12). Because the transformation efficiency of SL696 was very low, plasmid DNA was introduced into these strains by P22 transduction. We used the pNKB26-B6 plasmid, in which the small *Bam*HI C fragment of pNKB26 was deleted, to check the transformation efficiency of bacteria. Synthesis of *E. coli* O9 LPS was observed by the agglutination test with polyclonal anti-*E. coli* O9 rabbit serum.

Analysis of LPS by SDS-PAGE. LPS was extracted by the rapid small-scale method described previously (8, 23). Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) was carried out by the method of Tsai and Frasch (24). Silver staining of LPS was performed by the method of Hitchcock and Brown (5). The method for immunoblot analysis was that reported previously (7).

* Corresponding author.
† Present address: Department of Microbiology, Fukui Medical School, Matsuoka, Fukui 910-11, Japan.

TABLE 1. *S. typhimurium* strains

Strain	LPS type[a]	Relevant genotype	Origin or reference
SL696	S	*rfa*$^+$	26
SH5493	S	*rfe*	25
SL901	SR	*rfc*	26
his-515	Ra	*rfb rfaL*	13
TV119	Ra	*rfb*	1
TV163	Ra	*rfaL*	1
SL733	Rb_1	*rfaK*	St/22 (27)
SH5014	Rb_2	*rfaJ*	21
SH9156	Rb_3	*rfaI*	TV148 (22)
LB5010	Rc	*galE*	2
SL761	Rc	*galE*	*galE403* derivative of SL489 (22)
SH8572	RcP^-	*rfaP*	4
SL1032	Rd_1	*rfaG*	3
SL1181	Rd_2	*rfaF*	26
SL1102	Re	*rfaE*	26

[a] Types: S, LPS with the O-specific polysaccharide; SR, LPS with one repeating unit of the O-specific polysaccharide Ra to Re are as defined previously (1, 16).

RESULTS

Synthesis of *E. coli* O9 LPS in various mutant strains of *S. typhimurium*. When pNKB26 was introduced into *S. typhimurium* SL696, which has no defects in the genes for LPS synthesis, the bacteria agglutinated in anti-*E. coli* O9 rabbit serum (Table 2). This result suggested the presence of *E. coli* O9-specific polysaccharide on the surface of SL696 cells carrying pNKB26. The LPS preparation from this strain was analyzed by SDS-PAGE and immunoblot analysis (Fig. 1, lanes 1 and 2). Silver staining revealed the presence of two kinds of LPS. The presence of *E. coli* O9 LPS was confirmed by immunoblot analysis of the same sample. The ladder bands which reacted with anti-*E. coli* O9 rabbit serum in the LPS preparation of SL696 are shown (Fig. 1B, lane 2). The other bands showed the specificity of *S. typhimurium* by immunoblot analysis (data not shown). These results indicated that *E. coli* O9-specific polysaccharide was synthesized in SL696 and transferred to the R-core of *S. typhimurium* and that *E. coli* O9 LPS was efficiently transported to the cell surface of SL696.

TABLE 2. *E. coli* O9 LPS synthesis in *S. typhimurium*

S. typhimurium strain	Relevant genotype	Plasmid[a]			Agglutination with polyclonal anti-*E. coli* O9 rabbit serum
		pACYC184	pNKB26-B6	pNKB26	
SL696	*rfa*$^+$	+	ND[b]	+[c]	+
SH5493	*rfe*	+	+	+	−
SL901	*rfc*	+	+	+	+
his-515	*rfb*	+	+	+	+
TV119	*rfb*	+	+	+	+
TV163	*rfaL*	+	+	−	ND
SL733	*rfaK*	+	+	−	ND
SH5014	*rfaJ*	+	+	−	ND
SH9156	*rfaI*	+	+	−	ND
LB5010	*galE*	+	+	+	+
SL761	*galE*	+	+	+	+
SH8572	*rfaP*	+	+	+	+
SL1032	*rfaG*	+	+	−	ND
SL1181	*rfaF*	+	+	−	ND
SL1102	*rfaE*	−	−	−	ND

[a] Plasmids were introduced into each strain by transformation. +, Transformants obtained; −, failed to transform.
[b] ND, Not determined.
[c] pNKB26 was introduced into SL696 from SH5493(pNKB26) by transduction.

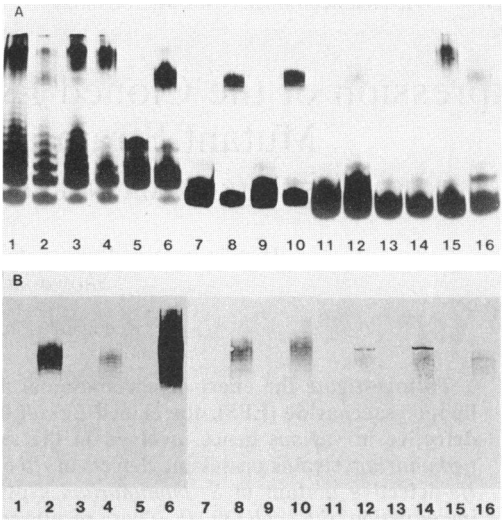

FIG. 1. SDS-PAGE of extracted LPS preparations from various strains of *S. typhimurium* carrying PNKB26. (A) Silver-stained gel; (B) immunoblot with anti-*E. coli* O9 serum. Lanes 1, SL696 LPS; lanes 2, LPS from SL696 carrying PNKB26; lanes 3, SH5493 LPS; lanes 4, LPS from SH5493 carrying pNKB26; lanes 5, SL901 LPS; lanes 6, LPS from SL901 carrying pNKB26; lanes 7, *his-515* mutant LPS; lanes 8, LPS from *his-515* mutant carrying pNKB26; lanes 9, TV119 LPS; lanes 10, LPS from TV119 carrying pNKB26; lanes 11, LB5010 LPS; lanes 12, LPS from LB5010 carrying pNKB26; lanes 13, SL761 LPS; lanes 14, LPS from SL761 carrying PNKB26; lanes 15, SH8572 LPS; lanes 16, LPS from SH8572 carrying pNKB26.

No strain of *S. typhimurium* carrying the *rfa* mutation could be transformed with pNKB26 except the *rfaP* mutant (Table 2). A transformant of TV163, an *rfaL* mutant with a complete R-core, also could not be obtained. However, pNKB26 could be introduced into *galE* and *rfaP* mutants, which possess the incomplete R-core, by transformation. Transformants of these strains, SL761, LB5010, and SH8572, agglutinated in anti-*E. coli* O9 rabbit serum (Table 2). Although these strains synthesized *E. coli* O9 LPS, the amount of *E. coli* O9 LPS produced by these strains was small (Fig. 1, lanes 11 to 16).

An *S. typhimurium his-515* mutant and strain TV119, possessing defects in the function of *rfb* gene cluster, were transformed with pNKB26. The *his-515* mutant and TV119 carrying pNKB26 agglutinated in anti-*E. coli* O9 rabbit serum (Table 2). SDS-PAGE and immunoblot analysis of LPS preparations from these strains showed the presence of *E. coli* O9 LPS (Fig. 1, lanes 7 to 10).

When pNKB26 was introduced into SL901, which is defective in *rfc*, the bacteria agglutinated in anti-*E. coli* O9 rabbit serum (Table 2). The SDS-PAGE profiles revealed that the LPS preparation from this transformant contained high-molecular-weight components possessing various lengths of the repeating units which could not be found in the LPS preparation from SL901 (Fig. 1A, lanes 5 and 6). These high-molecular-weight bands were shown to react with anti-*E. coli* O9 rabbit serum by immunoblot analysis (Fig. 1B, lanes 5 and 6). These results suggested that the low-molecular-weight bands corresponded to LPS possessing one

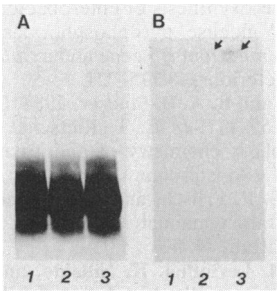

FIG. 2. SDS-PAGE of extracted LPS preparations from SL901 carrying plasmids pNKB26::γδ29 and pNKB26::γδ305. (A) Silver-stained gel; (B) immunoblot with anti-*E. coli* O9 serum. Lanes 1, SL901 LPS; lanes 2, LPS from SL901 carrying pNKB26::γδ29; lanes 3, LPS from SL901 carrying pNKB26::γδ305. Positions of *E. coli* O9-specific polysaccharide precursor are indicated by arrows.

repeating unit of the O-specific polysaccharide of *S. typhimurium* LPS and the high-molecular-weight bands corresponded to LPS possessing *E. coli* O9-specific polysaccharide. This result confirmed that the synthesis of *E. coli* O9-specific polysaccharide was independent of *rfc* and that the *rfc* product, O-repeating unit polymerase, was not essential for the synthesis of *E. coli* O9-specific polysaccharide chain consisting of a number of the repeating units (6, 11, 16).

Even after pNKB26 was introduced into SH5493, an *rfe*-deficient mutant, by transformation, the agglutination test of SH5493 carrying pNKB26 in anti-*E. coli* O9 rabbit serum was negative (Table 1). Silver staining revealed that the biosynthesis of *S. typhimurium* LPS was not influenced by the *rfe* mutation and that the ladder bands of *E. coli* O9 LPS were not found in the LPS preparation of SH5493 carrying pNKB26 (Fig. 1A, lane 4). However, immunoblot analysis showed the presence of a trace amount of LPS showing *E. coli* O9 specificity (Fig. 1B, lane 4).

Transfer of *E. coli* O9-specific polysaccharide from antigen carrier lipid to R-core of *S. typhimurium*. The question arose whether the *rfbT* product that functioned in the synthesis of *E. coli* O9 LPS in *S. typhimurium* was derived from *E. coli* O9 or *S. typhimurium*. To settle the question, pNKB26::γδ29 and pNKB26::γδ305, which were transposon insertion mutants of pNKB26 and regarded as *rfbT* mutants (9), were introduced into SL901. This *S. typhimurium* strain is defective in *rfc* but intact in the *rfaL* and *rfbT* functions and possesses complete R-core. LPS preparations from SL901 carrying these plasmids were analyzed by SDS-PAGE and immunoblot analysis (Fig. 2). SL901 carrying the mutant plasmids did not synthesize *E. coli* O9 LPS but only precursors with *E. coli* O9 specificity which were not bound to the R-core (Fig. 2B, lanes 2 and 3, arrows). These results were the same as those obtained when these mutant plasmids were introduced into *E. coli* K-12. It was suggested, therefore, that the presence of an intact *S. typhimurium rfbT* did not support the synthesis of *E. coli* O9 LPS, but intact *rfbT* function in the *E. coli* O9 *rfb* gene cluster was essential for the transfer and ligation of *E. coli* O9-specific polysaccharide to the R-core of *S. typhimurium* LPS.

DISCUSSION

Two gene blocks, termed *rfa* and *rfb*, specify the synthesis of the two polysaccharide parts of LPS, R-core and O-specific polysaccharide, respectively (Fig. 3) (for review, see

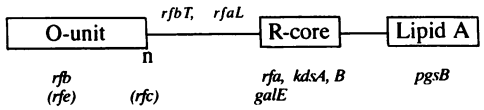

FIG. 3. Genes specifying the biosynthesis of each portion of LPS according to the review of Mäkelä and Stocker (11). Genes in parentheses are not common; *rfc*- and *rfe*-dependent LPS-synthetic pathways require *rfc* and *rfe*, respectively. *Salmonella* group A, B, D, and E strains belong to the former group and *E. coli* O8 and O9 strains and *Salmonella* group C1 and L strains belong to the latter group.

reference 11). The gene *galE* outside the main *rfa* cluster is also required to synthesize the R-core in *S. typhimurium*. The gene *rfb* specifies the synthesis of a unit of O-specific polysaccharide, and *rfc* polymerizes these units in *S. typhimurium*. Although there is little information about the mechanism of the synthesis of *E. coli* O9-specific polysaccharide, only *rfb* seems to direct the synthesis of polymerized O-specific polysaccharide. The requirement of *rfe* for the synthesis of *E. coli* O9 LPS is widely accepted (6, 11, 16), although the function of *rfe* is unknown yet. The O-specific polysaccharide synthesized independently of R-core bound to lipid A is transferred to R-core by the function of *rfaL* in the *rfa* gene cluster. The gene *rfbT* in the *rfb* gene cluster is also required to transfer the O-specific polysaccharide.

The *rfe*-deficient mutant of *S. typhimurium*, SH5493, harboring pNKB26 did not agglutinate in anti-*E. coli* O9 rabbit serum. This result agreed well with the report that introduction of an *rfe* mutation from *S. typhimurium* into an *E. coli* O9 strain prevented the synthesis of O9-specific polysaccharide (20). However, SDS-PAGE of the LPS preparation followed by immunoblot analysis, which is a highly sensitive detection method for the O-specific polysaccharide, revealed the presence of a trace amount of *E. coli* O9 LPS. There are two possible reasons for the presence of a trace amount of *E. coli* O9 LPS in SH5493. One is the leaky function of the *rfe* mutation of SH5493. The other is that the *rfe* genes of *E. coli* O9 and *S. typhimurium* differ in their functions. In fact, the *rfe* gene of *S. typhimurium* is different from that of *S. montevideo*, whose mechanism for the synthesis of LPS is similar to that of *E. coli* O9 in that the former lacks the gene for dTDP glucose pyrophosphorylase in the *rfe* region but the latter has it (10).

That transfer of *E. coli* O9-specific polysaccharide to the R-core occurred in *S. typhimurium* implied two possible mechanisms of transfer of the O-specific polysaccharide from the antigen carrier lipid to the R-core of LPS, as shown schematically in Fig. 4. One is the mechanism by which the *rfaL* and *rfbT* products of *S. typhimurium* transfer the synthesized *E. coli* O9-specific polysaccharides. The other is the mechanism by which the *rfaL* product of *S. typhimurium* worked with the *rfbT* product of *E. coli*. Namely, the *rfaL* product of *S. typhimurium* recognizes its own R-core and the *rfbT* product of *E. coli* O9 recognizes *E. coli* O9-specific polysaccharide on antigen carrier lipid. Then these two kinds of products from the different bacterial species work cooperatively and transfer *E. coli* O9-specific polysaccharide to the R-core of *S. typhimurium* LPS. We infer for the following reasons that the latter mechanism is actually operating in *S. typhimurium*. When the *rfbT* mutant plasmid was introduced into the *rfc* mutant strain of *S. typhimurium*, this strain could not transfer the synthesized *E. coli* O9-specific polysaccharide to the R-core but synthesized only precursor (Fig. 2). On the other hand, the *rfc* mutant of *S. typhimurium*

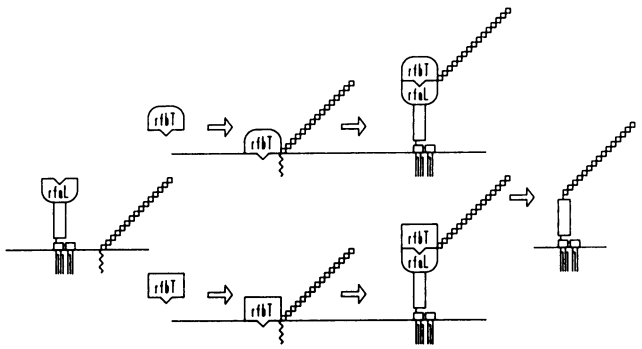

FIG. 4. Schematic representation of interaction between the *rfaL* and *rfbT* gene products. The *rfaL* and *rfbT* gene products (rounded shapes) are directed by the *S. typhimurium* gene. The *rfbT* gene product (rectangular shape) is directed by the *E. coli* O9 *rfb* gene. Pairs of small squares with seven feet represent lipid A, and the R-core is represented by an open rectangle; *E. coli* O9-specific polysaccharide is represented by the "staircase" of small squares.

carrying an intact *E. coli* O9 *rfb* synthesized *E. coli* O9 LPS. From these results, it was suggested that the *rfbT* product of *S. typhimurium* was unable to recognize *E. coli* O9-specific polysaccharide and failed to transfer O9-specific polysaccharide to the R-core. This also suggested the possibility that the *rfaL* product of *S. typhimurium* and *rfbT* product of *E. coli* O9 worked cooperatively for the synthesis of *E. coli* O9 LPS in *S. typhimurium*.

ACKNOWLEDGMENT

This study was supported by a Grant-in-Aid for Scientific Research (02670179) from the Ministry of Education, Science and Culture of Japan to N.K.

REFERENCES

1. **Beckmann, I., T. V. Subbaiah, and B. A. D. Stocker.** 1964. Rough mutants of *Salmonella typhimurium*. (2) Serological and chemical investigations. Nature (London) **201**:1299–1301.
2. **Bullas, L. R., and J. Ryu.** 1983. *Salmonella typhimurium* LT2 strains which are r^- m^+ for all three chromosomally located systems of DNA restriction and modification. J. Bacteriol. **156**:471–474.
3. **Enomoto, M., and B. A. D. Stocker.** 1974. Transduction by phage P1*kc* in *Salmonella typhimurium*. Virology **60**:503–514.
4. **Helander, I. M., M. Vaara, S. Sukupolvi, M. Rhen, S. Saarela, U. Zahringer, and P. H. Mäkelä.** 1989. *rfaP* mutants of *Salmonella typhimurium*. Eur. J. Biochem. **185**:541–546.
5. **Hitchcock, P. J., and T. M. Brown.** 1983. Morphological heterogeneity among *Salmonella* lipopolysaccharide chemotypes in silver-stained polyacrylamide gels. J. Bacteriol. **154**:269–277.
6. **Jann, K., and B. Jann.** 1984. Structure and biosynthesis of O-antigens, p. 138–186. *In* E. T. Rietschel (ed.), Handbook of endotoxins, vol. 1: chemistry of endotoxin. Elsevier Science Publishers B.V., Amsterdam.
7. **Kido, N., M. Ohta, K. Iida, T. Hasegawa, H. Ito, Y. Arakawa, T. Komatsu, and N. Kato.** 1989. Partial deletion of the cloned *rfb* gene of *Escherichia coli* O9 results in synthesis of a new O-antigenic lipopolysaccharide. J. Bacteriol. **171**:3629–3633.
8. **Kido, N., M. Ohta, and N. Kato.** 1990. Detection of lipopolysaccharides by ethidium bromide staining after sodium dodecyl sulfate-polyacrylamide gel electrophoresis. J. Bacteriol. **172**: 1145–1147.
9. **Kido, N., T. Sugiyama, T. Komatsu, T. Sekizaki, Z. B. Tong, M. Ohta, and N. Kato.** Submitted for publication.
10. **Lew, H. C., P. H. Mäkelä, H.-M. Kuhn, H. Mayer, and H. Nikaido.** 1986. Biosynthesis of enterobacterial common antigen requires dTDP glucose pyrophosphorylase determined by a *Salmonella typhimurium rfb* gene and a *Salmonella montevideo rfe* gene. J. Bacteriol. **168**:715–721.
11. **Mäkelä, P. H., and B. A. D. Stocker.** 1984. Genetics of lipopolysaccharide, p. 59–137. *In* E. T. Rietschel (ed.), Handbook of endotoxins, vol. 1: chemistry of endotoxin. Elsevier Science Publishers B.V., Amsterdam.
12. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
13. **Nikaido, H., M. Levinthal, K. Nikaido, and K. Nakane.** 1967. Extended deletions in the histidine-rough-B region of the *Salmonella* chromosome. Proc. Natl. Acad. Sci. USA **57**:1825–1832.
14. **Nishijima, M., and C. R. H. Raetz.** 1979. Membrane lipid biogenesis in *Escherichia coli*: identification of genetic loci for phosphatidylglycerophosphate synthetase and construction of mutants lacking phosphatidylglycerol. J. Biol. Chem. **254**:7837–7844.
15. **Raetz, C. R. H.** 1984. *Escherichia coli* mutants that allow elucidation of the precursors and biosynthesis of lipid A, p. 248–268. *In* E. T. Rietschel (ed.), Handbook of endotoxins, vol. 1: chemistry of endotoxin. Elsevier Science Publishers B.V., Amsterdam.
16. **Rick, P. D.** 1987. Lipopolysaccharide biosynthesis, p. 648–662. *In* F. C. Neidhardt et al. (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology, vol. 1. American Society for Microbiology, Washington, D.C.
17. **Rick, P. D., and M. J. Osborn.** 1972. Isolation of a mutant of *Salmonella typhimurium* dependent on D-arabinose-5-phosphate for growth and synthesis of 3-deoxy-D-mannoctulosonate (ketodeoxyoctonate). Proc. Natl. Acad. Sci. USA **69**:3756–3760.
18. **Rick, P. D., and M. J. Osborn.** 1977. Lipid A mutants of *Salmonella typhimurium*: characterization of a conditional lethal mutant in 3-deoxy-D-mannooctulosonate-8-phosphate synthetase. J. Biol. Chem. **252**:4895–4903.
19. **Sasakawa, C., K. Kawata, T. Sakai, S. Y. Murayama, S. Makino, and M. Yoshikawa.** 1986. Molecular alteration of the 140-megadalton plasmid associated with loss of virulence and congo red-binding activity in *Shigella flexneri*. Infect. Immun. **51**:470–475.
20. **Schmidt, G., H. Mayer, and P. H. Mäkelä.** 1976. Presence of *rfe* genes in *Escherichia coli*: their participation in biosynthesis of O antigen and enterobacterial common antigen. J. Bacteriol. **127**: 755–762.
21. **Stocker, B. A. D., M. Nurminen, and P. H. Mäkelä.** 1979. Mutants defective in the 33K outer membrane protein of *Salmonella typhimurium*. J. Bacteriol. **139**:376–383.
22. **Subbaiah, T. V., and B. A. D. Stocker.** 1964. Rough mutants of *Salmonella typhimurium*. (1) Genetics. Nature (London) **201**: 1298–1299.
23. **Sugiyama, T., N. Kido, Y. Arakawa, M. Mori, S. Naito, M. Ohta, and N. Kato.** 1990. Rapid small-scale preparation method of cell surface polysaccharides. Microbiol. Immunol. **34**:635–641.
24. **Tsai, C. M., and C. E. Frasch.** 1982. A sensitive silver stain for detecting lipopolysaccharides in polyacrylamide gels. Anal. Biochem. **119**:115–119.
25. **Valtonen, M. V., U. M. Larinkari, M. Plosila, V. V. Valtonen, and P. H. Mäkelä.** 1976. Effect of enterobacterial common antigen on mouse virulence of *Salmonella typhimurium*. Infect. Immun. **13**:1601–1605.
26. **Wilkinson, R. G., P. Gemski, Jr., and B. A. D. Stocker.** 1972. Non-smooth mutants of *Salmonella typhimurium*: differentiation by phage sensitivity and genetic mapping. J. Gen. Microbiol. **70**:527–554.
27. **Yamamoto, N., and T. F. Anderson.** 1961. Genomic masking and recombination between serologically unrelated phages P22 and P221. Virology **14**:430–439.