THE UNIVERSITY OF CALGARY

GENES AFFECTING MOTILITY IN *Salmonella typhimurium*

by

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A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE

DEGREE OF MASTER OF SCIENCE

DEPARTMENT OF BIOLOGY

CALGARY, ALBERTA

JANUARY, 1985

THE UNIVERSITY OF CALGARY

FACULTY OF GRADUATE STUDIES

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ABSTRACT

Salmonella typhimurium LT2 strains with smooth lipopolysaccharide or with lipopolysaccharide deficient in one or more core sugars (rough) were examined for motility, the presence of flagella and sensitivity to Chi, a flagellum - specific phage. Mot^+ strains with rough LPS did not swim as efficiently as smooth strains and had fewer flagella than smooth strains but showed no significant difference in Chi phage infectivity or propagation. Even strains with heptose - deficient lipopolysaccharide could be motile, for three deep rough strains were Fla^+ Mot^+, one strain was Fla^+ Mot^-; and another had no visible flagellar filaments (Fla^-). The gene responsible for the Mot^- phenotype could be co-transduced with the gene *cysE* and was designated *mot-402*.

An Rb2 chemotype strain (SL3748, *rfaI432*) also had a Mot^- phenotype which co-transduced with *pyrE* and *cysE* markers. The gene responsible for this phenotype was labelled *mot-401*, and represents a previously unreported locus controlling motility. This gene could not be complemented to give the Mot^+ phenotype by DNA from motile cells containing the genes between *pyrE* and *cysE*. A plasmid was constructed which contained a 27 kilodalton (Kd) insert of DNA from a *mot-401* strain which carried this gene as well as $pyrE^+$ and $cysE^+$ markers. This plasmid resulted in loss of motility of strains into which it was introduced, indicating

dominance of the mot-401 allele. The structure and number of flagella per cell were not affected by this gene.

Reversion of Mot^- cells to Mot^+ took place at a rate of approximately one in 10^7 cells, and is therefore likely due to a mutation in a single gene. Mot^+ revertants stay Mot^+. Growth curves of Mot^+ and Mot^- strains were similar, showing that the Mot^- phenotype is not the result of a general impairment in energy transduction of the cell. No consistent differences were found between Mot^+ and Mot^- strains when the protein profiles of inner and outer membranes were examined, and strains of both types appeared morphologically similar.

ACKNOWLEDGEMENTS

I would like to thank Professor K.E. Sanderson for his guidance and discussion throughout the course of this work.

I would also like to acknowledge the contributions of Ron Maclachlan, Dr. S. Kadam, Al Rehemtulla, and Jane Hurley through discussion and technical assistance, and recognize Lindsay Gwyn for preparation of growth media. Thanks also to Joyce Nelligan and Bob Maclean for help with electron microscopy and photography.

To Rita, Carl and Eric

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ABBREVIATIONS

amp^R	ampicillin resistant
ATP	adenosine triphosphate
bp	base pairs
cAMP	3'-5' cyclic AMP
CHe^-	non-chemotactic cells
CRP	cyclic AMP receptor protein
DOC	deoxycholate
EM	electron microscopy
EDTA	ethylenediamine tetraacetic acid
Fla^-	cells with no visible flagellar filament
g	grams
IM	inner membrane
kan^R	kanamycin resistant
Kb	kilobases
Kd	kilodaltons
KDO	3-deoxy-D-manno-octulosonic acid (dOclA)
L	litres
LPS	lipopolysaccharide
MG	minimal agar plus 0.2% glucose
min	minutes
ml	millilitres
MOI	multiplicity of infection
MOPS	3-N-morpholino propanesulfonic acid
Mot^-	non-motile phenotype; paralyzed flagella
PBS	phosphate buffered saline

Abbreviations (continued)

OM	outer membrane
PFU	plaque forming units
PMF	proton motive force
SDS	sodium dodecyl sulfate
str^R	streptomycin resistant
sec	seconds
tet^R	tetracycline resistant
TE	Tris-EDTA buffer
TM	total membrane fraction
Tris base	Tris(hydroxymethyl)aminomethane
Tris-HCl	Tris(hydroxymethyl)aminomethane hydrochloride
UV	ultraviolet
OD	optical density

I. INTRODUCTION

The lipopolysaccharide (LPS) structure of Gram - negative bacteria affects several properties of the outer membrane (OM) of these bacteria. Defects in the biosynthesis of LPS core oligosaccharides result in increased susceptibiliy of the organisms to antibiotics (Roantree et al., 1977), penetrability of lysozyme and sensitivity to bile salts (Sanderson et al., 1974), altered permeability of hemin (Janzer et al., 1981) and enhanced uptake of gentian violet (Hancock, 1984). Strains with mutations affecting core sugars therefore exhibit changes in the permeability of the OM. Rough strains also show changes in the following properties: colony morphology (Stocker and Makela, 1971), sensitivity to phage (Wilkinson et al., 1972; Lindberg, 1973), sensitivity to complement effects (Goldman and Austen 1975), maximal growth temperature (Chatterjee et al., 1976a) and degree of pathogenicity (Tannock et al., 1975; Roantree, 1967). In general, these defects are more striking as the length of core decreases. These properties indicate that the composition, structure, and stability of the OM are different for rough strains than for smooth. Proteins may undergo conformational changes or have different packing depending on the sugar composition of the core oligosaccharide, leading to a new OM organization and altered OM functions in LPS mutants (Gmeiner and Schlecht,

1980; Koplow and Goldfine, 1974). The protein composition of the OM is altered in deep-rough (Rd2 or Re) LPS core mutants (Ames et al., 1974; Koplow and Goldfine, 1974), and electron microscopic (EM) studies have shown that these types of mutation result in the loss of flagella at the cell surface (Irvin et al., 1975).

Flagella are also not synthesized under conditions of catabolite repression (Iino, 1977), in mutants defective in electron transport (Bar Tana et al., 1977), or at high temperature (Adler and Templeton, 1967). As a result of this non-flagellate (Fla^-) phenotype, bacteria subject to the above conditions are non-motile (Mot^-). Organisms with apparently normal flagella (Fla^+) may also be Mot^- for a number of reasons. Mutations in the *motA* and *motB* genes result in paralyzed flagella (Iino, 1977; Silverman and Simon, 1977), as do certain mutants of flagellar structural genes, *flaAII* and *flaQ* (Yamaguchi et al., 1972; Warrick et al., 1977). These genes are all found near unit 40 on the *Salmonella* genetic map (Enomoto, 1966a and b; Silverman and Simon, 1976; Sanderson and Roth, 1984). Heavy metal ions such as Cu^{++} can rapidly and completely inhibit motility (Adler and Templeton, 1967), as can anoxia (Khan and MacNab, 1980a), starvation (Berg et al., 1982; Manson et al., 1980), and imidazole reagents (Conley and Berg, 1984). A naturally occurring conjugative plasmid inhibits the motility of cells into which it is introduced (Bohlin and Burman, 1977).

As a result of the above information, I undertook a study to determine the relationship between LPS core structure, motility, and flagella in Salmonella typhimurium. Each strain was visualized with EM to determine numbers of flagella and was evaluated for motility by movement in semisolid agar and observation under the microscope. Rough strains were found to swim much less efficiently and possessed fewer flagella than smooth strains. Deep rough mutants were found which were motile and which possessed flagella.

Certain rough strains are also completely non-motile (Meyers et al., 1982). It was initially thought that a single mutation resulted in strains which had defects in LPS core biosynthesis (rfa mutants) and which were also Mot$^-$. P22 transduction of genes from these strains during my studies showed that the genes responsible for both these phenotypes were closely linked but could be separated. Conjugation of plasmids containing mot$^+$ alleles into the above Mot$^-$ strains did not reult in restoration of motility and a plasmid containing the mot$^-$ gene from one of these Mot$^-$ strains abolished motility in transconjugants, implying dominance of the mot$^-$ gene.

II. LITERATURE REVIEW

(A) The cell envelope.

Gram - negative bacteria are surrounded by a cell envelope composed of inner membrane (IM), OM and, between these, the periplasmic space and peptidoglycan layer (Costerton et al., 1974). Structures located outside this envelope include capsular polysaccharide, flagellar filaments, pili, protein layers (Lugtenberg and Van Alphen, 1983) and, in the Enterobacteriaceae, the enterobacterial common antigen (Makela and Mayer, 1976) which is in some strains attached to LPS.

(i) Functions of envelope components.

The IM contains phospholipids and proteins which play a role in the transport of nutrients, in oxidative phosphorylation, in the synthesis of phospholipids, peptidoglycan, LPS, and, via membrane-bound polysomes, of proteins (Lugtenberg and Van Alphen, 1983). Some proteins, such as receptors for chemotactic stimuli, are also responsible for transducing sensory stimuli across the IM (Boyd, et al., 1983; Parkinson, et al., 1983; Boyd and Simon, 1982). The integrity of the IM and the shape of the cell are maintained by the rigid peptidoglycan layer, and lipoproteins anchored to peptidoglycan as well as to OM create the periplasmic space, which contains a variety of hydrolytic and binding proteins (Lugtenberg and Van Alphen,

1983). The outer membrane is a barrier layer, retaining the proteins of the periplasmic space and preventing penetration of dyes, antibiotics, bile salts, and enzymes. Small hydrophilic molecules can cross this barrier and trace nutrients are taken up (Nikaido and Nakae, 1979). Zones of adhesion connect IM and OM membranes (Bayer, 1979).

(ii) Structure of the OM.

The structure of the OM has been best characterized in E. coli and S. typhimurium (Lugtenberg and Van Alphen, 1983). It constitutes an asymmetric bilayer with an outer leaflet containing LPS and protein and an inner leaflet containing predominantly phospholipids and protein (Figure 1). Proteins are for the most part integral and traverse the membrane (Osborn and Wu, 1980), covering about 59% of the outer surface of the outer membrane (Nikaido and Nakae, 1979). Most of these proteins form water filled channels which act as pores, allowing nutrients into the periplasmic space. LPS molecules are present in the outer membrane as monomers (Muhlradt et al., 1977), which are organized into separate domains (Lieve, 1977). These LPS molecules are held together by positively charged divalent ligands (Galanos and Luderitz, 1975; Lieve, 1974) and also form strong complexes with protein, which are seen in freeze-fracture electron micrographs as particles and pits (reviewed in Lugtenberg and Van Alphen, 1983).

(iii) Effect of mutations on OM structure

Mutants resulting in severely defective LPS (deep rough) have several changes in OM structure. Deep rough mutations result in considerable increases in the number of LPS molecules in the outer membrane (Gmeiner and Schlecht, 1979; Gmeiner and Schlecht, 1980), increases in the phospholipid content of the outer leaflet (Smit et al., 1975), and decreases in the protein content of the outer membrane (Ames et al., 1974; Koplow and Goldfine, 1974; Smit et al., 1975). In addition these mutations have a greatly increased sensitivity to hydrophilic compounds (Sanderson et al., 1974; Roantree, et al., 1977; Wilkinson et al., 1972) and excrete outer membrane blebs (Wensink and Witholt, 1981).

(B) The lipopolysaccharide (LPS).

LPS is an important molecule which comprises 30-40% by weight of the outer membrane (Osborn et al., 1972). The LPS monomers are large molecules (Figure 2) composed of an O-somatic antigen, a core saccharide, and an acylated oligosaccharide, termed lipid A. Biosynthesis and transport of the LPS molecule is a complex process requiring the co-ordinated expression of several genes; mutants defective in production of LPS have been well characterized through use in various studies of the structure, chemistry, genetics, and function of the LPS (Stocker and Makela, 1978; Makela and Stocker, 1981).

(i) O-antigen. The O-antigenic polysaccharide contains repeating tetrasaccharide units which often incorporate rare sugars and, in the case of Salmonella, enable species to be identified by their O-antigenic specificities (Wicken and Knox, 1980). Tetrasaccharide synthesis is directed by the genes of the rfb cluster at 44 units on the Salmonella genetic map and by the genes oafA, C, and R. These units are added to a lipid carrier, polymerized by the rfc gene product and attached to the LPS core by an O-translocase (the rfbT product). From 8 to 30 repeating units may be attached to any LPS molecule (Galanos et al., 1977).

(ii) Core. The LPS core is a sequence of sugars common to all Salmonella (Makela and Stocker, 1981) and is shown in Figure 2. Complete core is defined as the Ra chemotype LPS resulting from rfb mutations which are defective in O-chain biosynthesis or transport. Most of the structural genes for LPS biosynthesis lie in one or two clusters of genes at 79 units (Figure 5). Several of the genes in this cluster have been mapped (Makela and Stocker, 1981; Kadam et al., 1985 and have the order rfaGBIJ(KL)(CDE). Two genes, rfaP and rfaE, are located nearby at 77 units (Kuo and Stocker, 1972). Control of expression of the rfa genes is complex and involves the product of the rfaH gene at 84 units (Sanderson and Stocker, 1981). This gene is homologous to the sfrB gene of E. coli which produces a transcription antiterminator (Beutin et al., 1981).

Strains with mutations at galU (34 units) and galE (18 units) are unable to synthesize UDP-galactose and UDP-glucose, respectively. Since these compounds are substrates for two of the glycosyltransferases, the mutants make rough LPS of chemotype Rc and Rd1 respectively (Wilkinson et al., 1972).

(iii) Lipid A. The region of LPS which anchors the entire molecule into the outer membrane is the lipid A, which consists of phosphorylated glucosamine residues forming a disaccharide, to which are attached hydrophilic fatty acid chains. Charged phosphate residues in positions 1 and 4' have been implicated, along with similar residues on KDO in the core, in the stabilization of the OM by providing sites for divalent cation bridges between LPS monomers and proteins (Osborn, 1979; Osborn and Wu, 1980). Lipid A is responsible for the toxicity and pyrogenicity of LPS (Luderitz, 1977).

(C) Flagella and motility of Gram - negative bacteria.

(i) Mechanics of motility. Bacteria swim by the rotation of flagella, which are subcellular organelles that originate in the cell envelope and extend 15-20 micrometers from the cell surface (Silverman and Simon, 1977). Two types of motion are possible. During smooth swimming bacteria move in a straight line; tumbling produces rapid reorientations in the direction of swimming. The type of motion produced is determined by the direction of rotation of individual

flagella and, in bacteria with peritrichous flagella such as Salmonella, by the direction of rotation of all flagella contained within the flagellar bundle. Rotation with a counter-clockwise bias (CCW) produces smooth swimming while clockwise (CW) rotation results in dispersion of the flagellar bundle and tumbling (MacNab and Ornston, 1977). The direction of rotation of flagella, and therefore swimming motion, is influenced by chemical stimuli external to the bacterium so that cells will swim toward higher concentrations of attractants and away from higher concentrations of repellent (Parkinson, 1977). This chemotactic response therefore constitutes a behavioural system which has been analyzed at the genetic and molecular levels (Parkinson, 1977; Kehry and Dahlquist, 1983).

Three kinds of mutation result in the alteration or loss of swimming ability. Lesions in any of the numerous flagellar (fla) genes result in incomplete flagellar structures or in loss of flagella altogether; Fla^- strains have no visible flagellar filaments (Iino, 1977). Only four genes controlling motility (mot genes) have been described. (Iino, 1977; Dean et al., 1983). Mutations in these genes result in bacteria which have flagella that appear normal but which are paralyzed. More than eleven genes controling chemotaxis are known. In addition to these specific che genes, there are many genes responsible for the production of receptor proteins important in the chemotactic response.

Mutants in any of these genes are motile but do not respond to gradients of one or more chemoeffectors (Parkinson, 1977; Boyd and Simon , 1982).

(ii) Flagellar structure and synthesis. De Pamphilis and Adler purified intact flagella from E. coli (1971a) and determined their fine structure (1971b) and mode of insertion into the Gram - negative cell envelopes (1971c). Flagella are composed of a basal body, a hook region, and a filament, and mutations have been characterized in more than 30 genes which affect these structures (Iino, T., 1977; Silverman and Simon, 1977).

The basal body is the region of the flagellum in contact with the cell envelope (De Pamphilis and Adler, 1971c) (Figure 3). The M ring of the basal body is anchored in the IM while the S ring lies just above the periplasmic surface of the cytoplasmic membrane. The P ring is embedded in the peptidoglycan layer and the L ring is associated with LPS. In terms of biomechanics, the M and S ring form part of the flagellar motor, with the M ring corresponding to a rotor and the S ring a stator; in these terms the P and L ring would be bushings (Doetsch and Sjoblad, 1980). The functions of these sructures will be discussed at the molecular level in a later section.

At least 17 genes are responsible for the biosynthesis of complete basal body structures (Iino, 1977; Suzuki et al., 1978). As can be seen from Figure 4, specific functions

in biosynthesis have been assigned to only a few of these genes, with the result that little is known about the detailed structure of the basal body. Genes coding for proteins involved in the morphogenesis of basal bodies are located either in a cluster of flagellar structural genes at 23 units or in a cluster of genes containing fla, mot and che genes at 40 units on the Salmonella genetic map. Mutations in any of these genes can result in a Fla$^-$ phenotype.

The hook is a flexible structure joining basal body and filament, and is assembled from one principal type of protein monomer coded for by the flaFV gene (Iino, 1977). It allows the flagellar filament to occupy many positions relative to the cell body, making possible the formation of flagellar bundles (Spencer, 1984); this flexibility may also be important in the transfer of torque to the flagellar filament (Kato et al., 1984). Protein products of the flaV, flaU, and flaW genes are associated with the hook and are required for the assembly of the flagellar filament on the hook (Homma et al., 1984); strains with mutations in these genes are Fla$^-$.

Filaments are semirigid helices which self-assemble from flagellin protein monomers. In Salmonella, two types of flagellin are produced by the alternate expression of the H1 and H2 genes, which is controlled by the rh1 and hin genes (Zieg et al., 1977; Silverman and Simon, 1980; Bruist and

Simon, 1984). Mutations in H1 or H2 can result in a Fla$^-$ phenoype. The two flagellin molecules produced by these genes are functionally similar but antigenically distinct. Filaments transduce the energy of rotation of the flagellar motor into forward motion of the bacterial cell (Doetsch and Sjoblad, 1980). Helical transformations may occur in the filament of living bacteria, and may play a role in the mode of swimming of the bacterial cell in response to chemoeffectors (MacNab, 1978).

In addition to mutations already described, there are several conditions which can result in lack of flagellar formation. Glucose and a variety of catabolites repress synthesis of flagellar proteins (Adler and Templeton, 1967). The finding that strains defective in adenylcyclase (cya, 83 units) or in the cAMP receptor protein (crp, 72 units) were unable to synthesize flagella (Yokota and Gots, 1970) suggested that cAMP and the cAMP receptor protein (CRP) act at the same locus to positively regulate synthesis of flagellar genes. This gene was found to be flaI, at 40 units on the chromosomal map; the product of this gene acts to positively regulate expression of several other fla and mot genes (reviewed in Silverman and Simon, 1977). Ubiquinone mutants (ubiF, 14 units and ubiX, 46 units; Sanderson and Roth, 1984) are Fla$^-$, but it is not known whether these genes are regulatory or result in structural changes (Bar Tana et al., 1977). Elevated temperature can result in the

shutdown of flagellar synthesis (Adler and Templeton, 1967), and Yamamori et al., (1977) found a ribonucleic acid polymerase mutant of E. coli could not make flagella.

The state of the cell surface is important for flagellar assembly. Salmonella spheroplasts are unable to grow flagella in the presence of penicillin (Vaituzis and Doetsch, 1965), and deep rough LPS mutants of Salmonella as well as galU mutants of E. coli, are reported to be Fla$^-$ (Ames, et al., 1975; Irvin et al., 1975; Komeda et al., 1977).

(iii) Chemotaxis. Chemotaxis, a complex behavioural system, has been well reviewed by Parkinson (1977, 1981) and Boyd and Simon (1982). Motile cells exhibit responses to temporal gradients of attractants or repellents. Molecules of attractant or repellent interact with receptor proteins which may be periplasmic binding proteins such as the maltose binding protein (Brass and Manson, 1984), or integral IM proteins like the phosphotransferase system enzymes II (Pecher et al., 1983). Mutations affecting any receptor result in loss of response to only one attractant or repellent; all other chemotactic responses are normal.

Receptors interact with membrane bound signal transducers to generate a signal to the flagellar rotary motor (Boyd et al., 1983; Parkinson et al., 1983). A mutation in any one of the four genes (tar, tsr, trg, tap) coding for these transducer proteins results in loss of

response to more than one chemoeffector. After the signal has affected the direction of rotation of flagella at the level of the switch on the flagellar motor, a feedback mechanism involving the cheY and cheZ genes operates to influence activities of cheR, which produces a protein methylesterase, and cheB which produces a protein methyltransferase. This allows the signal transducer to again be stimulated by receptor protein.

The nature of the signal to the flagellar motor is not known. The signal must interact with the switch, which determines the direction of rotation of the flagella and which includes the protein products of the cheV and cheC genes (Boyd and Simon, 1982). Both cheV and cheC genes have alleles exhibiting Mot$^-$ (non-motile) and Fla$^-$ phenotypes. In additon, the proteins produced by these genes are structural components of the flagellar motor (Dean et al., 1983). These genes also known as flaAII and flaB are located within the cluster of fla genes at 40 units (Figure 5).

Mutations in two genes, cheA and cheW, result in a general non-chemotactic response (Che$^-$; Parkinson, 1981). Bacteria are motile but are altered in the relative amount of time spent in CCW flagellar rotation versus CW rotation. The cheA and cheW genes are contained in the mocha operon in E. coli with the motA and motB genes at 40 units, as shown in Figure 5 (Boyd and Simon, 1982).

(iv) Motility. Mutations in the motA and motB genes result in paralyzed flagella which are otherwise normal in appearance (Matsamura et al., 1977). Mutations in very specific regions of two flagellar structural genes, flaAII, and flaQ, result in the Mot^- phenotype; mutations in other regions of these genes can give rise to either Fla^- or Che^- phenotypes (Yamaguchi et al., 1972; Dean et al., 1983; Warrick et al., 1977).

The gene product of the motA gene is a 31,000 dalton protein and the product of the motB gene is a 39,000 dalton protein (Silverman and Simon, 1976). Both proteins are found exclusively in the IM (Ridgeway et al., 1977), but do not co-purify with flagellar basal bodies isolated using neutral detergents (Armstrong and Adler, 1969). EM observations of isolated basal bodies and SDS-polyacrylamide gel electrophoresis (SDS-PAGE) failed to detect differences between Mot^+ and Mot^- cells (Hilmen and Simon, 1976).

The use of E. coli and Salmonella mutants blocked in steps in oxidative phosphorylation plus use of uncouplers to oxidative phosphorylation has shown that proton-motive force (PMF) is the source of energy for flagellar movement (Thipayathasana and Valentine, 1974). In additon, certain components of ion flux are missing in Mot^- mutants (Szmelcman and Adler, 1976; Eisenbach, 1982; Manson et al., 1977; Manson et al, 1980; Khan and MacNab, 1980a and b). Block and Berg (1984) coupled the motB gene to a lacZ

promoter and introduced this into a motB$^-$ strain. They found that successive incorporaton of each motB protein resulted in a stepwise increase in the speed of flagellar rotation, and that each protein contributed the same increase in torque. The ratio of final to initial speeds gave an estimate of 16 proteins incorporated for the full motility of each flagellum. This number agrees with electron microscopic data indicating a 16-fold symmetry in the M-ring of the basal body (De Pamphilis and Adler 1971b), and suggests that the motB protein acts in the vicinity of the flagellar motor (Block and Berg, 1984). Since an intercistronic weak-complementation occurs in many combinations of motA$^-$ and motB$^-$ mutants (Enomoto, 1966a and b; Armstrong and Adler, 1967), it is possible that the products of these cistrons act as a structural complex. A model has been proposed in which a flux of protons is directed through channels probably composed of motA and motB proteins (Block and Berg, 1984; Berg and Khan, 1983). Interaction of these channel proteins with the M and S rings of flagella is thought to generate rotary force (Berg and Khan, 1983).

Production of motA and motB proteins is regulated by the flaI gene product in the same manner as other fla genes (Silverman and Simon, 1977). As well, a naturally occurring conjugative plasmid has been found which converts strains to

the Mot$^-$ phenotype when introduced into motile *E. coli* and *S. typhimuirium* strains (Bohlin and Burman, 1977).

III. MATERIALS AND METHODS

(A) Bacterial strains

The bacterial strains used are described in Table 1. Stock cultures of each strain were stored at -76 degrees C in L-broth with glycerol added to 15%. Strains were single colony isolated from frozen stocks, tested, and stored on L-agar slopes for routine use.

(B) Chemicals

The sources of chemicals used were as follows: acrylamide, N,N'-methylene-bisacrylamide, agarose (Type I), sodium dodecyl sulfate (SDS), 3-N-morpholino propane sulfonic acid (MOPS), Trizma-base, Trizma-HCl, ethidium bromide, silver nitrate (Sigma); phenol (Mallinkrodt); DNA molecular weight markers, proteinase K (Bethesda Research Laboratories); phosphotungstic acid (Fisher); L-(^{35}S)-methionine (Amersham); Omnifluor scintillant (New England Nuclear); and Cronex 6-plus X-ray film (Dupont).

(C) Media

Cells were routinely grown in L-broth (10 g Bacto-tryptone, 1 g glucose, 5 g Bacto yeast extract, 10 g NaCl, 3.5 mls 1M NaOH, 1 L water, pH 7.0). L-agar plates were made by adding Difco Bacto agar to a concentraton of 1.5%. Minimal medium contained 2 g/L KH_2PO_4, 1 g/L $(NH_4)_2SO_4$, 0.1 g/L $MgSO_4$. MG medium contained the above plus 20 g/L D-glucose as carbon source. Solid medium was made by

adding Difco Bacto agar at 1.5%. Medium was supplemented when necessary at the following concentrations: carbon sources, 0.2%; cysteine, 200 µg/ml; other amino acids, 50µg/ml; purines and pyrimidines, 50 µg/ml; tetracycline, 25 µg/ml, kanamycin sulfate, 75 µg/ml; sodium ampicillin, 50 µg/ml; and streptomycin sulfate, 200 µg/ml.

TTC motility medium contained 10g/L tryptone, 10 g/L peptone, 5 g/L NaCl, 0.16 g/L $Na_2HPO_4 7^{\cdot}H_2O$, 0.039 g/L sodium thiosulfate, 3 g/L Difco Bacto agar, 0.005% 2,3,5-triphenyl tetrazolium chloride, and was adjusted to pH 6.8 with 1.0 N HCl or NaOH. Plates were dried for 18 hours at 25 degrees C with lids removed to ensure uniform hydration and were stored for a maximum of two weeks at 4 degrees C in sealed plastic bags.

Chi medium contained per litre: Difco tryptone, 10 g; yeast extract 5 g; sodium glycerophosphate, 10 g; potassium lactate (50% w/v solution), 5 ml; $MnSO_4 4^{\cdot}H_2O$, 0.02 g; $MgSO_4$, 0.2 g; $FeSO_4 7^{\cdot}H_2O$ 0.02 g; glucose, 20 g; adjusted to pH 7.2. When plates were used, 1.5% Difco Bacto-agar was added.

(D) Conjugation methods

Conjugal transfer of plasmids was performed by adding 1 ml each of stationary phase donor and recipient cells to an L-agar plate. After mixing and overnight incubation, the cells were scraped off the plate, washed in sterile phosphate buffered saline (pH 7.2 - 7.4), and plated on selectve media.

(E) Bacteriophage methods

(i) Propagation. An overnight broth culture of sensitive cells was diluted 1:50 into L-broth; phage were added to a multiplicity of infection (MOI) of 2 to 5 for the temperate phages or 0.1 for virulent phages and grown at 37 degrees C ovenight with gentle shaking. After growth the phage lysates were cleared by centrifugation (12,000xg, 20 min., room temperature), filtered through a 0.45 μ filter, then stored at 4 degrees C over chloroform. Phage lysates were titred by the "drop on lawn" method of Gemski and Stocker (1967).

(ii) Transduction. The generalized transducing phage used for transduction of smooth strains was P22HT/1int; rough strains were transduced with ES18h1 or phage 19h1. The "drop-on-lawn" method of transduction was as follows: recipient strains were grown overnight in L-broth, lawns were prepared on plates of suitable selective medium, and drops of phage with a titre of 10^9 to 10^{10} PFU/ml were added to these lawns. Alternately 0.1 ml of diluted broth culture of the recipient strain was added to 0.1 ml of phage to give an MOI of 0.1. This mixture was then incubated at 30 degrees C for 30 min to permit phage adsorption, diluted 1:10 with L-broth, and 0.01 and 0.1 ml aliquots were plated onto selective medium. Growth was for 24 to 48 hours at 37 degrees C.

(iii) Phage sensitivity patterns. LPS type (smooth or rough) and the presence of functioning flagella were determined by the sensitivity of strains to specific phages. P22.c2, p22h.c2 and 9NA are specific for smooth S. typhimurium. Felix 0 (FO) is active on all smooth strains and on rfaL rough mutants (chemotype Ra). Phages 6SR, Ffm, Br60 and P22.c2 are rough specific. C21 is a rough specific phage active on mutants with LPS of chemotype Rc (galE), chemotype Rd1 (galU or rfaG) and rfaH mutants (Wilkinson et al., 1972). Chi phage attaches to and lyses cells with intact, functional flagella (Meynell, 1962). Overnight L-broth cultures of strains to be tested were used to prepare lawns of cells on L-agar plates. Drops of phage (approximately 10^8 PFU/ml) were placed on the lawn and plates were incubated at 37 degrees C for 4 to 8 hours.

(iv) Phage multiplication tests. The ability of strains to propagate Chi phage was tested in the following manner. A tube of Chi broth (4 ml) was inoculated with $2X10^6$ cells of the strain to be tested and $2X10^3$ PFU/ml of Chi phage. After 18 hours incubation at 37 degrees C, cellular debris was removed by centrifugation in a benchtop centrifuge (MSE Minor 35, full speed), and the resulting lysate was filtered through a 0.45 micron filter, and chloroform treated. Aliquots (0.02 ml) of hundred-fold dilutions of phage were spotted onto a lawn of an indicator strain (a motile strain, SA1355) and these plates were incubated 4 to 8 hours at 37

degees C before counting plaques under a dissecting microscope.

(F) Methods of measuring motility

(i) Microscopic methods. Strains to be tested were grown 4 hours in L-broth at 37 degrees C. Hanging drop preparations of these cultures were observed for motility at 400X under phase contrast.

(ii) Soft agar methods. Tubes or plates containing TTC motility medium were incubated with organisms from the surface of a plate or from a 4 hour L-broth culture and were incubated 24 to 72 hours at 30 degrees C. Growth of bacteria results in reduction of TTC in the medium so that the presence of bacteria can be easily visualized. Stabs of bacteria inoculated into TTC tubes were scored +, -, or $\pm$ for motility (see Figure 6a). When plates were used, a measurement was made from the original stab to the furthest point of visible growth and motility was expressed as the rate of movement of the bacteria in mm per hour.

(G) Electron microscopy

Stationary phase L-broth cultures of cells were centrifuged at full speed in a MSE Minor 35 benchtop centrifuge and the pellets were washed once then resuspended with distilled water. This suspension was mixed 4:1 with 1% phosphotungstic acid (pH 7.4) in a tube and transferred to a formvar coated grid. Negative stained bacteria thus obtained were observed with a Hitachi 300 electron microscope.

(H) LPS analysis

Cells obtained from L-agar plates were resuspended in PBS (0.2M phosphate buffer, pH 7.2, 0.9% NaCl) to give an OD_{640} of 1, then LPS was prepared by the method of Hitchcock and Brown (1983). One and one-half ml aliquots of the cell suspension were centrifuged for 2 min in a microfuge and the pellets were solubilized in 50 µl of lysing buffer (2% SDS, 4%β-mercapthoethanol, 1M Tris-HCl, pH 6.8 and 0.1% bromphenol blue). Cell suspensions were heated at 100 degrees C for 10 min, 25 µg of proteinase K was added in 10 µl lysing buffer and this mixture was incubated 120 min at 60 degrees C. These preparations were heated again at 100 degrees C for 5 min before running on 15% SDS-PAGE gels using the Laemlli system (Laemlli, 1970). Electrophoresis was done at 30 mA maximum current per gel and 200 V maximum voltage in Tris-glycine buffer, pH 8.30, with 0.1% SDS.

The LPS was visualized by silver staining using the method of Hitchcock and Brown (1983). Gels were fixed for a minimum of 18 hrs with 25% (v/v) isopropanol in 7% (v/v) acetic acid. LPS was oxidized for 5 min using 1.05 g periodic acid in 150 ml distilled water plus 4 ml 25% (v/v) isopropanol in 7% (v/v) acetic acid. Each gel was then washed eight times (30 min/wash) with 200 ml distilled water. Silver stain (28 ml 0.1N NaOH, 1 ml concentrated ammonium hydroxide, 5 ml 20% (w/v) silver nitrate, 115 ml distilled water) was added and reacted with the gel for 10

min. The gels were washed four times with 200 ml distilled water, then developed using 250 ml developer (50 mg citric acid monohydrate, 0.5 ml 37% formaldehyde in 1L distilled water). Develpment was stopped by washing gels for 1h with 200 ml distilled water plus 10 ml of 7% (v/v) acetic acid.

(I) Isolation of plasmid DNA

Plasmid DNA was isolated by the method of Maniatis et al. (1982). Strains to be tested were grown overnight on L-agar plates. Cells were scraped from 1 cm^2 of confluent growth and suspended in 100 µl of lysozyme buffer (50 mM glucose, 10mM EDTA, 25 mM Tris-HCl, pH 8.0 and freshly added lysozyme to 4 mg/ml). After 5 min incubation at room temperature, 200 µl of fresh, ice cold SDS buffer (1% SDS in 0.2 N NaOH) was added and mixed by gently inverting. This lysate was kept on ice for 5 min, then neutralized with 150 µl of cold potassium acetate solution (60 ml 5M potassium acetate, 11.5 ml glacial acetic acid, 28.5 ml water) and incubated on ice for 5 min. Cellular debris was pelleted by centrifuging lysates 5 min at 4 degrees C in a microfuge, and transferring the supernate to a fresh tube. An equal volume of phenol reagent (1 part phenol, 1 part 24:1 chloroform/isoamyl alcohol) was added to this supernate, the phases were mixed by shaking, then separated by centrifugation for 2 min at room temperature in a microfuge. The aqueous phase was transferred to a fresh tube and two volumes of 95% ethanol was added to precipitate DNA. This

was allowed to incubate 5 min at room temperature, DNA was pelleted by centrifugation for 5 min at room temperature in a microfuge, and 20 µl TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8) was added to resuspend DNA.

(J) Agarose gel electrophoresis of plasmid DNA

DNA in TE buffer was electrophoresed in 0.7% agarose in TBE buffer (89 mM Trizma base, 2.5 mM Na_2EDTA, 8.9 mM boric acid, pH 8.8) using a horizontal gel electrophoresis system. Electrophoresis was performed at 100 V constant voltage for 20h; DNA was stained with 0.5 µg/ml ethidium bromide in TBE buffer (pH 8.8), illuminated with a UV-transilluminator, and photographed (Polaroid Type 57 Land film) through a red filter.

(K) Transformation

One ml of a fresh overnight culture of bacteria was inoculated into 100 ml of L-broth and grown at 37 degrees C with shaking until the OD_{640} reached 0.2. Cells were chilled 15 min on ice and 1.5 ml volumes were centrifuged 4 min in a microfuge. The pellets were washed once with 1.5 ml of 0.1 M $MgCl_2$, then were suspended in 0.75 ml of 0.1 M $CaCl_2$ in 50 mM MOPS buffer (pH 6.5) and incubated 40 min on ice. These suspensions were centrifuged, resuspended in 0.3 ml volumes of solution containing 50 mM $CaCl_2$, 50 mM $RbCl_2$, and chilled for 5 min. Plasmid DNA was added to 0.1 ml volumes of these cells and the suspensions were chilled on ice for 30 min. A heat shock was carried out at 42 degreees C for 2 min,

followed by incubation on ice for 10 min, dilution of cell suspensions 1:20 with pre-warmed L-broth, and incubation at 37 degrees C for 2 hours. Cells were then plated on suitable selective medium.

(L) Protein analysis

Strains were grown overnight at 37 degrees C in minimal medium, diluted 1:10 in 20 ml fresh prewarmed medium, and grown to an OD of 0.3. Labelling of proteins was accomplished by adding ^{35}S-methionine to a concentration of 3 to 5 μCi/ml and allowing cells to grow to late log phase. Cells were pelleted by centrifugation at 8000xg for 5 min, washed once with 10 mM Tris-HCl, pH 7.5, resuspended in 2 ml 10 mM Tris-HCl, pH 7.5, and sonicated 60s on ice. Lysates were centrifuged 8000xg for 10 min, the supernatants were transferred to ultracentrifuge tubes, and centrifuged 50 min at 110,000xg in a Beckman 50 Ti rotor. The pellets were resuspended in 100 ul 10 mM Tris-HCl, pH 7.5. Sample buffer (200 mM Tris-HCl, pH 6.8; 3% w/v SDS; 30% v/v glycerol; 5% v/v 2-mercaptoethanol, and 0.02% bromophenol blue) was added to 50 μl of this suspension, and this total membrane, or TM, fraction was boiled for 10 min. Membranes were separated by the method of Whitfield, et al. (1985). Eighty μl of fresh 1% Sarkosyl in 10 μM Tris-HCl, pH 7.5, was incubated with the remaining 50 ul of cell suspension for 30 min at room temperature, this mixture was centrifuged 5 min at 30 psi (95,000 rpm; 140,000xg) in a Beckman airfuge, and the

supernate was poured into a fresh tube. An equal volume of sample buffer was added to the supernate, which contained isolated IM, and 75 µl of sample buffer was added to resuspend the pellet, which contained the OM. Both suspensions were boiled for 10 min, and stored at 4 degrees C until use. Radioactivity was quantified by scintillation counting using a Beckman LS 8000 liquid scintillation counter. Samples (0.05 ml) were applied to squares of Whatman filter paper, allowed to dry, then counted in 10 ml Omnifluor scintillant. Proteins were resolved by SDS-PAGE by the method of Laemlli (1971), using 12% (w/v) acrylamide resolving gels and 5% acrylamide stacking gels and electrophoresing at 2 mA per well until the tracking dye reached the end of the gel. Gels were stained with Coomassie brilliant blue, destained, and dried under vacuum. Dried gels were immediately subjected to autoradiography by exposure against Cronex 6-plus X-ray film at -70 degrees C. Films were developed according to manufacturers instructions.

IV. RESULTS

(A) Motility, Chi phage sensitivity, and flagella of known smooth and rough strains.

Several standard smooth and rough LPS strains were examined for motility by microscopic observation and by inoculation to TTC semisolid motility agar (Table 2). Motile cells were seen in microscopic observation of all strains except SL3748 and SA1377. In all cases where motile cells were seen, motility was vigorous with a preponderance of smooth swimming cells over cells which were tumbling. The estimated percentage of motile cells per total bacteria viewed did not decrease with decreasing LPS core saccharide chain length, but instead seemed to be related to strain-specic factors. Therefore, SL3748, with an Ra chemotype LPS, had fewer motile cells than any other strain (with the exception of the two completely non-motile strains) and SA34, which has the Re LPS chemotype, appeared to have a completely motile population of cells.

Tests using TTC motility medium in tubes (Table 2, Fig. 6a) confirmed the above observations. Information from quantitative motility test on TTC agar plates (Table 2, Fig. 6b) indicates that these organisms can be placed into three groups based on motility. The first group, smooth strains, has average range of movement through semi-solid agar greater than 2.0 mm per hour, the second group comprises

rough strains which move at less than 1.0 mm per hr. and the third group contains strains which are completely non-motile. Within the second group the rate of movement does not depend absolutely on the length of LPS core saccharide (LPS chemotype). Strain SL3749, which has the Ra LPS chemotype, also has the slowest rate of movement while SL3750 (Rb2 chemotype LPS) has the fastest. There appears to be a general impairment of swimming efficiency or swimming speed among rough strains when compared with smooth strains, but the final rate of swimming seems also to be partly due to strain-specific factors.

Chi phage attaches to and will propagate in bacteria with normal flagella and motility (Meynell, 1961), Salmonella mutants which are non-motile because of abnormal flagellar filaments (Iino and Mitani, 1967), and bacteria with flagellar filaments sheared to a length of 70 nm (Schade et al., 1967). The flagellar basal body is likely the receptor (Yamaguchi and Fujita, 1977), and data collected by Ravid and Eisenbach (1983) indicates that incessant flagellar rotation may be necessary for adsorption of the phage. Chi phage lysis and propagation was therefore used as an indicator of the presence of functioning flagella.

The data for Chi phage propagation by and lysis of standard strains is summarized in Table 3. All strains except SL3748 and SA1377 were lysed by the phage and gave a

titre of the phage at least 10^6 times greater than the infecting dose. Two strains were not lysed by Chi phage; SL3748 was non-motile and did not support multiplication of the phage, SA1377 was non-motile but allowed propagation of the phage to wild-type levels.

Electron microscopy was used to visualize flagella on cells of each strain (Figs. 7 and 8). On average there are fewer flagella present on rough strains, though the average numbers of flagella per cell and histogram patterns do not show a decrease in flagellar content with decreasing core saccharide length. Flagella are present on the two non-motile strains, SL3748 and SA1377, in numbers comparable to other motile strains.

(B) Motility of Re strains

Ames et al., (1974) found that deep rough mutants of Salmonella typhimurium with the Re LPS chemotype were completely non-motile. Studies done previously in this lab (Meyers et al., 1982) also suggested deep rough strains were non-motile.

(i) Characterization of the Re chemotype. Several previously isolated putative Re chemotype strains (Sanderson et al., 1974; Lindsay et al., 1973) were therefore tested for LPS chemotype by sensitivity to rough or smooth-specific phages, growth in the presence of deoxycholate (DOC), and migration of LPS on SDS-PAGE (Table 4; Figure 9). Chemotype Re strains are lysed by Ffm phage but not by C21, which is

specific for chemotypes Rc and Rd1, or P221, which lyses all other rough chemotypes (Wilkinson et al., 1972); these strains are also sensitive to DOC. All strains in Table 4, with the exception of SL3770, are Re by these criteria. Figure 9 shows on SDS-PAGE gel of these strains compared to several standard rough chemotype strains; again all are of Re chemotype. The strain SA35 shown in this photograph had been characterized as Re by phage and DOC sensitivity tests (Sanderson et al., 1974) but LPS did not co-migrate with the LPS of an Re standard strain. It is therefore important to confirm LPS chemotype by SDS-PAGE.

The motility and Chi phage sensitivity of these confirmed Re strains was determined (Table 5). Three of these strains had the motility characteristic of rough strains, which was defined in the previous section, and had the ability to propagate Chi phage at wild-type levels. The two remaining strains were non-motile by all methods tested but differed in the ability to propagate Chi phage, suggesting some differences in flagella. Electron microscopy of these and other Re strains (Figure 10) shows that SA575 differs from all other strains in that it has no visible flagella and is therefore by definition a Fla$^-$ strain. Strain SA1377 has flagella but no motility, therefore it is Mot$^-$; the gene responsible was designated mot-402. Circumstantial evidence at this point indicated that this

mot-402 gene was separate from mutants determining the Re chemotype LPS determined by the mutation *rfaC630*.

(ii) Separation of *rfaC* from *mot-402*. Transductions were done with P22 phage obtained from SA1377 by UV induction into recipients with pyrimidine or cysteine auxotrophies. All recombinants were selected for prototrophy, tested for phage sensitivity to determine LPS type, and tested for motility (Table 6). Crossover diagrams were drawn for each type of transduction (Figure 11).

Results from crosses using SA2443 as the recipient strain show that the wild type phenotype comprises the largest class of recombinants and that rough motile strains account for most of the remainder. Recombination events resulting in both rough and non-motile phenotypes were very rare, and no strain was found which had a smooth non-motile phenotype. Genes were ordered from *cysE* based on frequencies of transduction with the *cysE*$^+$ marker, where higher frequencies of transduction were taken to indicate closer linkage and the class showing the lowest frequency of co-transduction was assumed to be the double-crossover event. The gene order, reading toward *pyrE*, is therefore *cysE rfaC630 mot-402*; separation of the gene controlling motility from the gene resulting in the Re LPS chemotype has been demonstrated.

A similar cross was done using SA2436, a *pyrE*$^-$ strain. All recombinants were *pyrE*$^+$, 26 of 31 were smooth and

motile, 5 of 31 were rough and motile, and no recombinants were either smooth, non-motile, or rough, non-motile. A cross-over diagram can be constructed from these data (Figure 11b) which shows that mot-402 can be separated from rfaC630 but which implies a reversal of the the order of genes compared to the previous experiments.

Recombinants with a rough phenotype were tested for sensitivity to the smooth and rough-specific phages and for deoxycholate sensitivity. All rough strains were identified as having the Re chemotype by these methods; four of these rough mot^+ recombinants were confirmed to have Re LPS (data not shown).

(C) Transductions involving the mot-401 gene.

(i) Separation of rfaI432 and mot-401 alleles. One strain, SL3748, has been found which carries the rfaI432 mutation (has Rb_3 chemotype LPS) and which is completely non-motile (Meyers et al., 1982). Generalized transducing phages ES18.h1 and 19.h1 were used to transduce genes from this strain into normally motile, smooth recipient strains which were $cysE^-$ or $pyrE^-$

Strain SA2436, which carries the pyrE123 mutation and is fully motile, was used as a recipient in six crosses (Table 7), and all four classes of recombination event were represented in the results. Smooth motile strains constituted the majority class while rough motile, and rough non-motile transductants were present in nearly equal

numbers. Only one smooth non-motile transductant was found in all six crosses. The crossover diagram for these crosses (Figure 12) shows that the logical gene order is pyrE$^-$ rfaI432 mot-401, assuming that the rarest event will be the double-crossover. Transductions were performed with the same phage lysates used for the above crosses; the fully motile cysE$^-$ auxotrophs SA2443 and SA2568 were used as recipients. Results using both transducing phages and both recpient strains were similar and are pooled in Table 8. The majority class of transductants contains the smooth, motile strains, which are 79% of total. Other classes have roughly similar numbers of transductants within the error of the method, a finding which allows genes to be ordered pyrE-(rfaI 432, mot-401)-cysE (Figure 13). Some transductions resulted in recovery of only three classes of transductants, so that either rough motile or smooth non-motile transuductants were not represented. The mot-401 gene is transferred 57% of the time with rfaI 432, indicating a linkage close enough to make recombination between these genes relatively rare in any single experiment.

Phage P22 grown on a smooth non-motile strain obtained from one of the above crosses (SA2939) was used to transduce SA2443 to prototrophy. There was no transfer of the mot-401 gene in four experiments yielding 228 transductants; in the three remaining experiments non-motile strains accounted for 17 of the 121 transductants (14%).

Recipient strains used in the above transductions, as well as strains representative of most classes of transductant, were tested for motility by microscopic observation plus quantitative tests on TTC motility agar plates; some non-motile smooth strains were also examined for the presence of flagella (Table 9). The two smooth non-motile strains, SA2939 and SA2940, retained levels of flagella comparable to their parent strains. Impairment of motility is therefore not caused by decreasing the numbers of flagella present on bacterial cells. The rough non-motile strain, however, had reduced numbers of flagella. Presumably this is the result of the transfer of the rfaI 432 gene into SA2443, and raises interesting questions about the relationship between the structure of the OM to the proper biosynthesis and stability of flagella.

Experiments which used the "drop-on-lawn" method of transduction had as a control the portions of the bacterial lawn which remained uninoculated with phage. Prototrophic revertants of recipient strains were seen relatively rarely and, when found, were motile. Several transductions were done by the other method outlined in Materials and Methods, which involved plating mixtures of phage and recipient bacteria to selective medium. In order to include a control for spontaneous mutations to non-motility, loops of broth which contained the recipient bacteria used were streaked for single colonies on L-agar plates. Testing of 273 single

colony isolates of SA2443, 140 isolates of SA2568 and 100 isolates of SA2436 showed that all were motile. Non-motile strains seen after transduction therefore did not arise from mutation.

(iii) Failure to transduce a *mot*$^-$ strain to *mot*$^+$. A strain carrying Tn10 near *pyrE* (SA2950) was constructed using a P22 generalized transducing phage to transduce genes from SA2703 into SL3770 selecting for tetracycline resistance. The Tn10 is about 67% linked to *pyrE*$^+$ and is probably between *pyrE* and the *rfa* cluster. In a similar fashion, a strain carrying Tn10 near *cysE* (SA2953) was constructed by transduction of the element from SA2715 into SL3770. The Tn10 is approximately 50% linked to *cysE*.

Generalized transducing phage ES18.h1 was grown on each of these strains and used to transduce SL3748 according to the "drop-on-lawn" protocol in Material and Methods. Crosses using SA2950 as the donor resulted in 2 of 31 (6%) smooth non-motile transductants and 29 of 31 (94%) rough non-motile transductants. When SA2953 was the source of genes, 11 of 49 (22%) transductants were smooth non-motile and 38 of 49 (78%) were rough non-motile. No motile transductants were found. The *rfaI*$^+$ allele is 6% linked to the Tn10 near *cysE* and 22% linked to the Tn10 near *pyrE*, values which agree closely with linkages previously obtained for these Tn10 elements with *pyrE* and *cysE*.

(D) *Dominance of the mot-401 allele over mot*$^+$.

(i) Complementation experiments using plasmids pKZ26 and pKZ27. Plasmids have been constructed which carry known genes of the *rfa* cluster (Kadam et al., 1985). The plasmid pKZ26 has a Hind III insert containing *rfaGBIJ*. These plasmids were transformed into four *mot*$^-$ strains, namely SL3748 (*rfaI 432 mot-401*), SA1377 (*rfaC630 mot-402*), SA2939 (*rfa*$^+$ *mot-401*), and SA2940 (*rfa*$^+$ *mot-401*), in an attempt to obtain *mot*$^+$ transformants. The presence of the above plasmids was ensured by selection for ampicillin resistance (Ap^R); motility and LPS type were tested as unselected markers. Plasmids pKZ26 and pKZ27 had been made by insertion of DNA fragments into pBR322. pBR322 was therefore also transformed into all four recipient strains as a control to determine whether plasmid-encoded genes could affect motility. Selection in this case was for Ap^R, tetracycline resistance (Tc^R), motility, and LPS type.

Motility was not restored by addition of any of these plasmids. Both pKZ26 and pKZ27 complemented the *rfaI 432* mutation of SL3748, giving the resulting strain an Rfa$^+$ phenotype. No changes other than acquisition of antibiotic resistance were noted for the other strains. It is not certain whether these plasmids carry the *mot*$^+$ alleles of these mutants; since negative results on complementation were observed, no conclusions can be drawn.

(ii) Complementation tests using pKZ3. pULB113 (RP4::mini Mu), obtained from Van Gijsegem and Toussaint

(1982), was used by Kadam et al., (1985) to construct an R' plasmid which carries about 80 Kb of Salmonella chromosomal DNA and which complements cysE, pyrE and several rfa mutations. This plasmid, pKZ3, therefore carries the region of the chromosome betweeen pyrE and cysE and should definitely carry the mot$^+$ allele; plasmid encoded genes specify Tc^R, Ap^R, and kanamycin resistance (Km^R) The strain from which the chromosomal genes were derived, SA2386 (pyrE 123 recA1) was tested and found to be fully motile. Transfer of pKZ3 from SA2535 into SA2954 (pyrE 123 mot-401 recA1) was accomplished by conjugation, selecting for the plasmid and against the multiply auxotrophic parent which carries the plasmid by using MG medium with added antibiotics. This also imposed a selection for chromosomal genes on the plasmid by adding a requirement for transfer of pyrE$^+$, preventing the breakdown of pKZ3.

Strains obtained by the above conjugation were tested for motility in TTC tubes and for the presence of the plasmid by agarose gel electrophoresis (Figure 14). Motile strains were never found after conjugation even though the majority of strains carried the intact pKZ3 plasmid (Figure 14), showing that the mot-401 allele is dominant to the mot$^+$ allele carried on pKZ3.

(iii) Construction of hybrid plasmids carrying the mot-401 allele. The mot-401 allele was obtained on plasmids in order to more fully examine the property of dominance of

the Mot$^-$ phenotype, as follows. Strain SA2876 served as the conjugation donor of pULB 113 (RP4:: miniMu) into SA2941 (*ilvA mot-401*), selecting for the Km^R, Tc^R, and Ap^R markers carried on the plasmid. Agarose gel electrophoresis confirmed that the plasmid was contained in SA2962, the strain produced by this manipulation (data not shown). The plasmid was then mobilized by conjugation from SA2962 into either SA2389 (*cysE1709 pyrE125 rec A1*) or SA2386 (*pyrE123 recA1*) selecting for Km^R, Ap^R, and prototrophy by growth on MG agar containing antibiotics. R- primes are formed as a result of random Mu - mediated integration and excision from the *Salmonella* chromosome (Van Gijsegem and Toussaint, 1982) and only some of the R- primes formed would result in prototrophy and growth of the transconjugant. Plasmids carrying the region of the chromosome including *pyrE*$^+$ and *cysE*$^+$ would complement the *pyrE*$^-$ and *cysE*$^-$ mutations of SA2389 to allow growth. Plasmids incorporating *pyrE*$^+$ would complement the *pyrE*$^-$ defect of SA2386. The *recA*$^-$ mutation present in both recipients should lower the incidence of loss or breakdown of the plasmid through recombination.

Transconjugants that had been single colony isolated on selective medium were used for preparation and agarose gel electrophoresis of plasmid DNA. Plasmids from several strains had lost the band on the gel corresponding to pULB113, which is 62 Kb, and had gained a larger plasmid band which ran close to or above the pSLT band which is 90

Kb (Figure 15). All of the strains containing these larger R - primes were Mot$^-$ while strains which received only pULB113 were Mot$^+$ (data not shown). The mot-401 allele must therefore be carried on the chromosomal DNA inserted into these plasmids, must be between pyrE$^+$ and cysE$^+$, and must be dominant. Two plasmids thus constructed, namely pKZ35 in SA2948 and pKZ37 in SA2973, were selected for further study. Plasmid sizes can only be roughly estimated from agarose gels but probably range from about 85 Kb for the plasmid designated pKZ35 and about 130 to 140 Kb for the plasmid designated pKZ37 (Figure 15). EM observations showed that SA2948, though Mot$^-$, had an average of 12.3 flagella per cell compared to 12.4 flagella per cell on the parent, SA2389.

(iv) Transfer of pKZ35 into SA572. SA2948 was used as the conjugation donor of pKZ35 into SA572 (pyrE strA201), which is Mot$^+$ and resistant to 1000 µg per ml streptomycin (str^R). Selection of transconjugants was accomplished by using MG agar plus tetracycline, kanamycin, ampicillin, and 1000 ug per ml streptomycin. Only cells of SA572 which had gained the plasmid grew on this selective medium. All transconjugants were Mot$^-$ and the presence of pKZ35 could be demonstrated in several transconjugants one is shown in Figure 15 despite the fact that SA572 is not Rec A$^-$.

(E) Reversion of mot$^-$ to mot$^+$

Smooth strains carrying the mot-401 allele, such as SA2939, SA2940, and SA2948, all show areas of motility in tubes or on plates of TTC motility medium if incubated at 30 degrees C more than 30 hours. SL3748 never showed this reversion but a S. typhimurium LT7 sister strain carrying the same rfaI and mot$^-$ alleles did revert within 48 hours to give motile cells. It was necessary to distinguish whether reversion was a genetic event in specific cells, occurring at a finite rate, or whether all cells have the same tendency to show motility when incubated in motility medium. Strains to be tested were grown 5 hours in L-broth at 37 degrees C with shaking, then a loopful of this culture was streaked to the surface of a TTC motility agar plate. This plate was incubated for up to 48 hours and observed frequently for areas of motility. The results shown in Figure 16 are typical of rfa$^+$ mot-401 strains in general. The time at which motile revertants became apparent ranged from 30 to 48 hours even when streaks were made from the same broth of the same organisms. Obviously, specific cells revert to Mot$^+$ while others do not. It was not possible to estimate the number of cells required for reversion to Mot$^+$ sine the number of cells on the plates was not determined. When motile cells from these plates were streaked to TTC motility medium, uniform growth covered the plate in under 24 hours. Standard motile strains gave the same result.

In a second experiment, 5 hour cultures of rfa$^+$ mot-401 cells which had been in L-broth at 37 degrees C with shaking, were diluted in L-broth. These dilutions were then added to sterile petri dishes in 0.1 ml aliquots and pour plates were made using TTC motility agar, effectively suspending individual cells within the agar. After the agar had hardened, plates were incubated for up to 72 hours at 30 degrees C. Counts of colonies allowed quantitation of the initial number of cells present on the plate, and the plates were observed for reversion to motility. Lower dilutions (up to 10^{-4}) showed motility very early, in under 30 hours. Higher dilutions (10^{-6}, 10^{-8}) showed areas of motility in 30 to 48 hours. Figure 17 is a photograph of 0.1 mo of a 10^{-7} dilution of SA2940 in TTC motility agar after 40 hours growth. Motile cells have spread from a single area on the plate, implying a single reversion event. At various times during growth, colonies were carefully picked up in a pasteur pipette, then resuspended and diluted in L-broth. Duplicate 0.1 ml volumes of each dilution were spread on L-agar, incubated 24 hours at 37 degrees C, and colonies counted. A viable cell count was also done when motile revertants were first apparent on a plate. When the number of cells in each colony is plotted vs. time, an estimate can be made of the number of cells present when reversion takes place. From Figure 18, it can be seen that motility is visible on the plate at about 40 hours, or at a count of

about 2×10^7 cells per colony. Reversion to motility must have taken place at some time before this, but the frequency of the event would still be less than 1 in 10^6. Reversion is therefore a genetic event, occurring in specific cells at a defined rate.

(F) Further examination of the Mot^- phenotype.

(i) Membrane proteins. The protein profiles of IM, OM, and total membranes (TM; a mixture of IM and OM) were obtained for several Mot^- and a Mot^+ strain using the protocol for membrane isolation and SDS-PAGE described in Materials and Methods. Figure 19 shows the results of one such experiment, in which the membrane protein profiles of three smooth Mot^- strains from different sources are compared with the protein profiles of a smooth Mot^+ strain. One very faint band with a weight of approximately 55 Kd appears in the IM of SA2944 (Rfa^+ Mot^-) but is not seen in the IM's of the other Rfa^+ Mot^- strains. This band is not due to the *mot*$^-$ genotype. Two bands in the OM of SA2963, (Rfa^+ Mot^+) both of which are about 90 Kd, appear to be present in a higher concentration in this strain than in the three Rfa^+ Mot^- strains. This difference was not seen in a second gel run with the same strains at the same time (data not shown). A band of approximately 50 Kd is much more pronounced in OM fractions of SA2939 and SA2975 than in the other two strains, but since it is not present in SA2944 (*rfa*$^+$ *mot-401*), it is not due to the *mot-401* gene.

A second experiment compared the two *rfa*$^+$ *mot*$^-$ strains, SA2939 and SA2940, to an *rfa*$^+$ *mot*$^+$ strain (SA2963) as well as SL3748 (*rfaI432* *mot-401*). No differences could be seen between the protein profiles of SA2963 and SL3748 by the methods used (data not shown). If there are differences between the protein content of Mot$^+$ and Mot$^-$ strains, they cannot be resolved by the procedures used.

(ii) Growth and cell morphology. A growth curve experiment was performed with smooth Mot$^+$ and Mot$^-$ strains, namely SA2963, SA2443, SA2939, and SA2940. No differences in growth rate were found (data not shown), indicating that there are no gross differences in metabolism and energy production between strains. The amount of growth after 5 hours in L-broth, observed visually, appeared similar for numerous other Mot$^+$ and Mot$^-$ cells. Mot$^+$ and Mot$^-$ cells were the same size and looked morphologically similar when viewed by light or electron microscopy.

V. DISCUSSION

(A) Influence on motility and flagella of LPS core mutations.

Motility of the strains examined depends in a general way on LPS chemotype, in that rough strains do not swim as efficiently as smooth strains. Six of the eleven strains used in this part of the study are isogenic lines carrying different *rfa* mutations which were constructed by Roantree et al. (1977; see Table 1). These six strains, SL3748, SL3749, SL3750, SL3769, SL3789 and SL3770, showed large differences in both motility and numbers of flagella (Figure 8, Table 2). Some flagellar function must be impaired possibly at the level of the interaction between the L-ring and the LPS. Although no specific function has yet been assigned to the L-ring, it is possible that impairment of motility and decrease in flagellar numbers in *rfa* mutants could be due to increasing fluidity of the OM with decreasing core length or to the rearrangement of OM structure in response to these mutations.

The fluidity of the OM has been estimated by electron spin resonance spectroscopy using spin labelled fatty acids as a probe (Rottem and Lieve, 1977), by the rate of diffusion of newly synthesized LPS over the cell surface (Muhlrad et al., 1974), and by measurement of lateral

diffusion of phospholipids, LPS and proteins using the 'fluorescence redistribution after photobleaching' technique of Schindler et al. (1980a, b). Experiments were performed mainly with smooth, Rc chemotype, and deep rough strains. Though results obtained varied widely with the technique used, they indicated that OM's of rough strains are more fluid than those of smooth strains. If this increased fluidity were responsible for impairment of swimming and loss of flagella, Rd2 and Re mutants should show the least motility and the fewest flagella. This is not the case. Table 2 shows that the strain with complete core (Ra chemotype) has the least motility, and Figure 8 shows that Rb3 chemotype strains have the fewest flagella.

Rearrangements of OM structure could also result in impaired function of flagella. Several lines of data indicate that such rearrangement takes place in the OM of rough strains. Freeze-fractures of smooth cells seen under the electron microscope show very small fracture faces densely covered with particles which create corresponding pits in the inner fracture face (Irvin et al., 1975). Deep rough strains have OM's which are much more planar (Irvin et al., 1975), due to a reduction in total proteins in the OM (Gmeiner and Schlecht, 1979) as well as loss of specific proteins and concomitant increase in the relative number of phospholipid and LPS molecules present (Koplow and Goldfine, 1974; Ames et al., 1974). Changes in relative protein

content of strains with chemotypes Ra through Rb3 have also been found to correlate with an increase in LPS content in the OM (Gmeiner and Schlecht, 1979) and the OM's of Rc mutants fracture more easily than those of wild-type strains, again indicating a more planar structure (Smit et al., 1975).

Such structural differences could lead to changes in LPS-protein or protein-protein interactions in the OM which are necessary for the full activity of specific proteins in the L-ring of flagella. Core sugars of LPS seem to be necessary for adsorption of phage K3 to reconstituted vesicles containing outer membrane protein A (OmpA; Van Alphen et al., 1979), and the formation of a hexagonal lattice structure by the outer membrane protein C (OmpC) is dependent on the presence of core oligosaccharide (Yamada and Mizishuma, 1980). Certain LPS core structures could also be necessary for the proper conformation and function of flagellar proteins, and specific protein-protein interactions might also be changed. Since the arrangement and packing of the OM could be quite different for each rfa mutant chemotype, only some chemotypes would exhibit the maximum loss of flagella and motility.

Other factors known to influence rate of swimming of bacterial cells are temperature (Schneider and Doetsch, 1977) and the presence of amino acids in the growth medium at concentrations of 10mM or greater (Nossal and Chen, 1973;

de Jong et al., 1977). Motility tests in TTC motility medium were always performed at 30 degrees C and were done each time with all strains using the same batch of medium. Flagellar synthesis was not catabolite repressed under the growth conditions used, since smooth strains produced large numbers of flagella even though 110 mM (0.2% w/v) glucose is included in L-broth. 280 mM (0.5% w/v) glucose is sufficient for repression of flagellar synthesis in E. coli (Adler and Templeton, 1967).

Chi phage was capable of infecting and propagating in all strains except SL3748. Sensitivity to Chi phage indicates that flagellar motor activity and rotation of flagella (Ravid and Eisenbach, 1983) or of flagellar hooks (Kagawa et al., 1984) is occurring. This is surprising only in the case of SA1377, which is completely non-motile and is not visibly lysed by Chi, though the phage can propagate in this strain. The reason for this is not known.

(B) Motility of deep rough strains.

The LPS chemotype of mutant strains can be directly and effectively confirmed using SDS-PAGE analysis (Fig. 9; Munford et al., 1980; Hitchcock and Brown, 1983). Confirmed Re chemotype strains were shown to be capable of motility under the conditions used, could propagate Chi phage, and had significant numbers of flagella (Table 5; Fig. 10), contradicting previously published results for other Re strains (Ames et al., 1974; Koplow and Goldfine, 1974).

Flagella are absent from deep rough strains under conditions where divalent cations are omitted from the growth medium, but can be seen when $MgCl_2$ is included (Irvin et al., 1975). Other cations (Ca^{++}, and Na^{+}), as well as sucrose, can decrease the permeability of the OM of Re strains to a number of periplasmic proteins (Chatterjee et al., 1976b) as well as to antibiotics and gentian violet (Stan-Lotter et al., 1979), presumably because of a rearrangement of the OM. The presence of flagella on strains used in this study may be a consequence of the medium used in their growth, since L-broth contains a relatively high concentration of NaCl. The accumulated data again indicate that a particular OM organization is necessary for the biosynthesis and correct insertion of flagella into the cell wall and that the effect on motility of LPS mutations is mediated through changes in OM organization.

Transductions in which DNA from SA1377 was introduced into recipient strains clearly show that the rfaC630 genotype is not responsible for the Mot^{-} phenotype of the parent, since $Rfa^{-}Mot^{+}$ recombinants were obtained. The presence of a gene specifically controlling motility (mot-402) is implied, though the location of the gene could not be pinpointed due to the frequencies of transduction obtained. This mot-402 gene was never co-transduced with pyrE, was co-transduced rarely with cysE, and did not seem to be closely linked to rfaC630. In fact, though I have

shown the gene to lie between pyrE and cysE in the crossover diagrams (Fig. 11), it could be between units 78 and 79 on the map near the location of the mem gene. If this were the case, though, $Rfa^{+}Mot^{-}$ recombinants would be expected at a relatively high frequency in crosses using SA2443 as the recipient strain. These were not found. Measurement of the frequency of co-transduction of rfaC630 with pyrE and cysE constitutes an internal control on the validity of the crosses; these frequencies were very close to those given in the literature (Sanderson et al., 1974).

(C) mot-401 is responsible for the Mot^{-} phenotype of SL3748 and its derivatives.

It was initially thought that the rfaI432 mutation of SL3748 was responsible for both the Rb3 LPS chemotype and for the Mot^{-} phenotype of this strain. Strong evidence for this hypothesis come from the fact that SL3748 and a sister S. typhimurium LT7 strain (SL3784) were constructed by transferring $pyrE^{+}$ from the parent, TV148, (Roantree et al., 1977). Both recipients received the rfaI432 allele and also became Mot^{-}. It seemed that the Rfa^{-} and Mot^{-} phenotypes were transferred together, perhaps by transfer of a single gene. Kadam et al. (1985) have shown that the $rfaI^{+}$ allele is responsible for the production of galactosyltransferase I, an enzyme which adds α-1,3-galactose to the glucose I unit onto the main chain oligosaccharide of the LPS core. It seems very unlikely that this protein would have a role in

the flagellar motor in the IM. Another possibility is that the OM structure characteristic of Rb3 chemotype strains results in loss of motility by modifying or eliminating necessary protein-protein or protein-LPS interactions in the OM. A strain carrying a second rfaI allele (SL3810), however, is motile though it has the same Rb3 chemotype as SL3748.

This problem was resolved when the transductions described in section (C) of the Results showed that the rfaI432 allele could be separated from the gene controlling motility and that this new gene, mot-401, was between pyrE and cysE near the rfa cluster. The gene is closely linked to rfaI432 and it could be successfully transferred from Rfa^+ Mot^- parents into Mot^+ recipients. The location of this gene at unit 79 clearly separates it from the other mot genes, which are found at unit 40, although the phenotypes are similar. There is no previously described gene near unit 79 which would result in a Mot^- phenotype, and no corresponding gene has been discovered in E. coli (Bachmann, 1983).

There was some concern that the Mot^- phenotype could arise from a mutation at some other point in the recipient chromosome at a frequency which would make it appear to be a product of transduction. Testing of prototrophic revertants from "spot-on-lawn" transductions and testing of single colony isolates of the recipients used in other

transductions never turned up Mot$^-$ mutants. The existence of the mot-401 allele at unit 79 was therefore accepted.

An attempt was made to transduce the Mot$^+$ phenotype from strains carrying Tn10 elements near pyrE$^+$ and cysE$^+$ into Mot$^-$ recipients. These attempts were not successful. Selection was necessarily for Tc^R, since the recipients were pyrE$^+$ and cysE$^+$, and the Tn10 element may not have been closely linked to the mot-401 gene, lowering the chances of co-transduction of Tn10 and mot-401. If the mot-401 gene were dominant over mot$^+$, the chances of obtaining mot$^+$ transductants would also be low, though the introduction of the mot$^+$ gene into the chromosome through recombination and the subsequent loss of mot-401 DNA should result in a small number of Mot$^+$ transductants. A final possibility is that there is no mot$^+$ allele expressed by the same gene as the mot-401 allele. mot-401 would then be a gene similar to the gene found by Bohlin and Burman (1977), which is carried on a naturally occurring conjugative plasmid and which abolishes motility in strains into which it is introduced.

(D) Significance of the dominance of the mot-401 allele.

The fact that the mot-401 allele was not complemented by the pKZ3 plasmid strongly suggested that mot-401 is dominant to the mot$^+$ allele (if mot$^+$ exists). This was confirmed by obtaining mot-401 on a plasmid and transferring it into Mot$^+$ strains, making these strains Mot$^-$. One of the

plasmids constructed, pKZ35, was about 85 Kb in size; since pULB113 is 62 Kb in size, the chromosomal insert must be about 23 Kb. Genetic mapping experiments using ES18 transduction (Kuo and Stocker, 1972) and conjugation of the F factor into the cluster of rfa genes (Sanderson and Saeed, 1972a) gave data which allow the map interval between pyrE and cysE to be calculated. This distance is about 0.6 min (Sanderson and Roth, 1984) to 0.85 min (Kadam et al., 1985), corresponding to a linear distance of from 26 Kb to 38 Kb. The size of the chromosomal insert in pKZ35 is at the lower end of these values, though it is able to complement both pyrE$^-$ and cysE$^-$ defects. The insert must carry little or no DNA outside this region, and must therefore carry mot-401 between these markers.

The dominance of mot-401 could arise in one of two ways. This gene could produce a protein which interferes with transcription of the region containing the motA and motB genes. There is recent evidence that flaD defects prevent expression of the mocha operon of E. coli and the motA and motB genes of Salmonella specifically (Kagawa et al., 1984). This type of mutation is recessive. If the protein produced by the mot-401 gene was able to interact with either the wild-type gene product or with the promoter of the motA and B genes in a way which halted expression of this operon, the result would be a dominant Mot$^-$ phenotype. The mot-401 gene could also produce a protein which

interacts in some way with the flagellar motor to disrupt generation of the rotary force of the flagellum. Such a protein could interfere with the transport of protons through channels formed by motA and motB gene products, despite the energy made available by proton translocation, or interfere directly with the flagellar proteins mechanically involved in rotation of the flagella. To act in this manner, the mot-401 gene product would have to interact with, or be inserted in, the IM.

The dominance of mot-401 may explain why the gene has not been found by other researchers despite an intense interest in motility. Most protocols for obtaining Mot^- strains involve selection for Mot^- mutants from a motile population of cells, for example by successive transfer of subpopulations of mutagenized cells from the centre of plates of semisolid agar (Dean et al., 1983). All protocols in use would select only recessive mot^- mutations, in which the production of defective protein results in loss of motility. Dominant Mot^- strains cannot be obtained by selection, they must be found in nature, since selection is for a mutation in a structural gene and would be recessive.

(E) The reversion of mot^- to mot^+

The reversion of mot^- to mot^+ created a problem in deciding which strains to call Mot^- and which to call Mot^+. Only SL3748 and SA1377 did not revert upon prolonged incubation in liquid or on semisolid medium, though a sister

strain to SL3748 which presumably carried the same mot-401 gene (SL3787, Roantree et al., 1977) did show reversion. The reason for the stability of the Mot^- phenotype of SL3748 is not known. It seems unlikely that this stability is due to the rfaI 432 mutation also present in this strain or to the LPS chemotype determined by this mutation since SL3787 carries the same mutation and has the same LPS chemotype. The stability of the Mot^- phenotype in SL3748 must be ascribed to the presence of a different genetic background in this strain.

All other Mot^- strains produced Mot^+ variants after a certain period of growth. The random times at which such revertants arose as well as the fact that only a very small proportion of cells reverted indicated that reversion was a genetic event. Motile revertants very quickly took over the tubes or plates where they arose. It was found that Mot^+ revertants were rarely encountered after growth of Mot^- strains in L-broth for 5 hours with shaking. These conditions result in growth of most strains to late log or early stationary phase, or a concentration of about 1×10^9 cells per ml of medium. These growth conditions were therefore used for testing of the quantitative motility, numbers of flagella, and other phenotypes associated with Mot^- strains. Recipients in transduction, conjugation, and transformation were often tested for motility initially from solid medium. Strains which were fully motile always

appeared motile under these conditions and no strain designated Mot$^-$ after testing this way was later found to be Mot$^+$, though revertants did occasionally show up.

Motile cells arising from populations of Mot$^-$ bacteria have been described by Bohlin and Burman (1977), though in their case Mot$^+$ cells may have resulted from the loss of the plasmid carrying the dominant mot$^-$ gene. An unstable flaAIII mutant isolated by Dean et al., (1983) gave rise to a spotty spread on swarm plates and to occasional motile cells in liquid cultures, a phenotype similar to the one I see with Mot$^-$ cells. Finally, Iino and Enomoto (1971) have described a phenomenon which looks the same as my Satellite colonies (see Figure 6a) which they attributed to leaky motility mutants. It is possible that the reversion phenomenon seen in this study is similar to the above situations and that it arises from conditions or mutations inactivating the mot-401 gene or its gene product.

(F) Other phenotypes of Mot$^-$ cells.

No differences in the proteins of IM or OM were found between Mot$^+$ and Mot$^-$ strains which could be ascribed to the mot-401 gene. The protein product of this gene might be located in the cytoplasm, interacting with the genome or with the flagellar motor at the cytoplasmic side of the IM. It also could be present in the IM or OM, but in levels too low to be detected by the methods used. This was found to be

the case for the motA and motB gene products (Hilmen and Simon, 1976; Silverman et al., 1976).

All other characteristics of Mot$^-$ cells seemed the same as those of Mot$^+$ strains, including rate of growth, cell morphology, and size. It seems unlikely, therefore, that there are any differences in metabolic function between Mot$^+$ and Mot$^-$ cells. In this respect, the Mot$^-$ phenotype caused by the mot-401 gene is similar to the phenotype resulting from mutations in other mot genes.

(G) Future experiments.

Plasmids containing the mot-401 gene should be transferred by conjugation into strains containing rfa mutations. Complementation of rfa genes by these plasmids would confirm the location of mot-401. Curing of such plasmids from strains should result in reversion of the resulting cells to Mot$^+$, thereby proving that the Mot$^-$ phenotype is caused by a gene carried on the plasmid.

The mot-401 gene can be cloned from pKZ35 into another plasmid vector, perhaps pBR322, using a shotgun method and selecting for loss of motility in strains into which the plasmid is introduced. The protein product of this gene could then be determined by minicell analysis (as described in Rehemtulla, 1984) and its cellular location found. This would narrow the possibilities for the place and mode of action of the gene product. It may be interesting to perform heteroduplex analysis of this gene with other mot genes,

including motA, motB, and the gene on the plasmid discovered by Bohlin and Burman (1977), to see if there is any homology. Plasmids containing the mot-401 gene could also be used as DNA hybridization probes to identify other strains containing the gene and get some idea of the distribution and importance of this gene in bacterial populations.

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Table 1. The strains used in this study.[a]

Strain	Genotype	Source/Reference
G30	galE	Lopes et al., 1972
SA 26	proA26 tre$^+$ clb$^+$ rfaE629 HfrB2 (P22)$^+$	K.E. Sanderson
SA 33	proA26 tre$^+$ clb$^+$ gal-446 rfaC630 (P22)$^+$ F$^-$	Sanderson et al., 1974
SA 34	proA26 tre$^+$ clb$^+$ gal-446 rfa-631 (P22)$^+$ F$^-$	K.E. Sanderson
SA 35	proA26 tre$^+$ clb$^+$ gal-446 rfa-632 (P22)$^+$ F$^-$	Sanderson et al., 1974
SA 572	metA22 trpE2 hisF1009 strA201 xyl-1 ilvA99 pyrE123 malB111	K.E. Sanderson
SA 575	rfa-543	B.A.D. Stocker
SA 1355	rfa$^+$ prototrophic (P22)$^+$	Chatterjee et al., 1976a
SA 1377	rfaC630 (P22)$^+$	Sanderson et al., 1974
SA 1648	rough mutant	Schmidt and Luderitz, 1969[b]
SA 1649	metA22 trpE2 H1-b H2-e,n,x flaA66 rpsL120 xyl-404 metE551 rfaE543	Wilkinson et al., 1972
SA 1970	metA22 trpC2 hisF1009 rpsL201 xylR1 recA1 srl-202::Tn10 (P22-sensitive)	K.E. Sanderson
SA 2386	pyrE123 recA1	Kadam et al., 1985
SA 2389	cysE1709 pyrE125 xyl-3 recA1 srl-202::Tn10	Kadam et al., 1985
SA 2436	pyrE123	K.E. Sanderson
SA 2443	cysE396	K.E. Sanderson
SA 2535	pyrE123 recA1/pKZ3 cys$^+$ pyr$^+$	Kadam et al., 1985
SA 2568	cysE2	K.E. Sanderson
SA 2703	zhj1404::Tn10	K.E. Sanderson

Table 1. (continued)

Strain	Genotype	Source/Reference
SA 2715	zhj1416::Tn10	K.E. Sanderson
SA 2876	metA22 metE551 ilv-452 galE436 hsdLT6 hsdS29 strA120 xyl-404 trpAB126/pULB113$^+$	van Gijsegem and Toussaint, 1982
SA 2939	cysE$^+$ mot-401	This study
SA 2940	cysE$^+$ mot-401	"
SA 2941	ilvA::Tn10 mot-401	"
SA 2942	pyrE123 mot-401	This study[c]
SA 2944	rfa$^+$ mot-401 zhj1404::Tn10	This study[d]
SA 2948	cysE1709 pyrE125 xyl-3 recA1 srl-202::Tn10 carries pKZ35	This study
SA 2950	rfa$^+$ zhj1416::Tn10	"
SA 2953	rfa$^+$ zhj1404::Tn10	"
SA 2954	rfa$^+$ pyrE123 mot-401 recA1::Tn10	This study[e]
SA 2958	rfaC630 mot$^+$	This study
SA 2959	rfaC630 mot$^+$	"
SA 2960	rfaC630 mot$^+$	"
SA 2961	rfaC630 mot$^+$	"
SA 2962	ilvA::Tn10 mot-401/pULB113$^+$	"
SA 2963	rfa$^+$ mot$^+$	"
SA 2971	cysE$^+$ rfaI432 mot-401	"
SA 2973	pyrE123 recA1 carries pKZ37	"

Table 1. (continued)

Strain	Genotype	Source/Reference
SA 2974	*cysE*$^+$ *mot*$^+$ *rfaI432*	This study
SA 2975	*cysE*$^+$ *rfa*$^+$ *mot-401*	"
SL 3748	*rfaI432*	Isogenic lines derived by transduction of *pyrE*$^-$ to *pyrE*$^+$ using ES18 phage grown on *rfa* mutants with linked transfer of *pyrE-rfa*. (Roantree et al., 1977)
SL 3749	*rfaL446*	
SL 3750	*rfaJ417*	
SL 3769	*rfaG471*	
SL 3770	*rfa*$^+$	
SL 3789	*rfaF511*	
SL 3810	*pyrE125 rfaI738*	Roantree et al., 1977

[a] All strains are *Salmonella typhimurium* LT2 unless otherwise noted.

[b] This is a *Salmonella minnesota* strain.

[c] This strain was derived by transduction, using P22, of SA 2703 genes into SA 2436, selecting for a Tc^R *pyrE*$^-$ strain. (This strain was not stocked.) This strain was used as a donor of genes through P22 mediated transduction into SA 2939, resulting in SA 2942.

[d] This strain was constructed by P22 mediated transduction of genes from SA 2953 into SL 3748.

[e] This strain was constructed by P22 mediated transduction of genes from SA 1970 into SA 2942.

Table 2: Motility of standard smooth and rough strains.

Strain	LPS genotype[a]	LPS chemotype	% cells motile in microscope[b]	Motility in TTC tubes (30° C)[c]	Motility on TTC agar plates: rate of movement (mm/h)[d]
SL 3770	rfa$^+$	smooth	99	+	3.0
SA 1355	rfa$^+$	smooth	100	+	2.24
SL 3749	rfaL 446	Ra	20	±	0.12
SL 3750	rfaJ 417	Rb2	100	+	0.72
SL 3748	rfaI 432	Rb3	0	-	0
SL 3810	rfaI 738	Rb3	90	+	0.68
G 30	galE	Rc	50	+	0.36
SL 3769	rfaG 471	Rd1	50	+	0.32
SL 3789	rfaF 511	Rd2	100	+	0.60
SA 1377	rfaC 630	Re	0	-	0
SA 34	rfa-631	Re	100	+	0.40

[a] A partial genotype is given. The complete genotype may be found in Table 1.

[b] This number is an estimate based on the observation of at least three fields of view. Both smooth-swimming and tumbling cells were included.

Table 2 (continued)

[c] Motility was scored as positive if there was diffuse growth within the tube away from the stab with a corresponding reduction of TTC in the media (red colour). Motility was scored as negative if growth and reduction of TTC were confined to the stab line (see Figure 6a). ± indicates that discrete, well-formed colonies were apparent some distance from the stab, but there was no diffuse growth characteristic of completely motile strains.

[d] At 25 h, the furthest distance of growth and reduction of TTC from the original stab was measured and the rate of movement calculated. A uniform rate of movement was assumed. Growth was at 30°C.

Table 3. Chi phage sensitivity of and propagation by standard smooth and rough strains.

Strain	LPS genotype[a]	LPS chemotype	Lysis by Chi phage[b]	Propagation of Chi phage: titre after growth (PFU/ml)[c]
SL 3770	*rfa*$^+$	smooth	+	5.5×10^{10}
SA 1355	*rfa*$^+$	smooth	+	4.2×10^{10}
SL 3749	*rfaL* 446	Ra	+	8.0×10^{10}
SL 3750	*rfaJ* 417	Rb2	+	2.5×10^{10}
SL 3748	*rfaI* 432	Rb3	-	4.5×10^{2}
SL 3810	*rfaI* 738	Rb3	+	8.0×10^{11}
G 30	*galE*	Rc	+	1.1×10^{10}
SL 3769	*rfaG* 471	Rd1	+	4.3×10^{9}
SL 3789	*rfaF* 511	Rd2	+	8.0×10^{10}
SA 1377	*rfaC* 630	Re	-	1.5×10^{10}
SA 34	*rfa*-631	Re	+	5.9×10^{11}

[a] This is only a partial genotype. The full genotype can be found in Table 1.

[b] Drops of Chi phage (10^8 PFU/ml) were applied to lawns of each strain on L-agar, Chi agar, and EMBO + ys agar. These plates were incubated at 37°C for 4 to 8 h, then read. Results were the same for each type of medium used. (EMBO + ys agar was 37.5 g EMB agar, 5.0 g yeast extract, 5.0 g NaCl, 1L water.)

Table 3 (continued)

[c] Chi phage was obtained by the method for phage propagation given in Materials and Methods. Drops of the phage lysate thus obtained (0.02 ml/drop) were then applied to lawns of SL 3770 prepared on Chi agar.

Table 4. Tests to determine the LPS chemotype of deep rough strains.

Strain	LPS genotype[a]	LPS chemotype determined by SDS-PAGE[b]	Lysis by phage FO ; Ffm	C21	P221	Growth on L-DOC agar[c]
SL 3770	rfa$^+$	smooth	+	-	-	+
SA 26	rfaE 629	Re	-	+	-	-
SA 33	rfaC 630	Re	-	+	-	-
SA 34	rfa-631	Re	-	+	-	-
SA 575	rfa-543	Re	-	+	-	-
SA 1377	rfaC 630	Re	-	+	-	-

[a] This is a partial genotype only. The full genotype is contained in Table 1.

[b] As shown in Figure 9.

[c] Cells from cultures grown overnight in L-broth at 37°C were streaked on plates containing L-agar + 0.4% deoxycholate (DOC; w/v). These plates were then incubated overnight at 37°C and examined for growth.

Table 5. Motility and Chi phage sensitivity of, and propagation by, deep rough strains.

Strain	LPS chemotype[a]	% cells motile in microscope[b]	Motility in TTC tubes (30°C)[b]	Motility on TTC agar plates: rate of movement (mm/h)[b]	Lysis by Chi phage[c]	Propagation of Chi phage: titre after growth (PFU/ml)[d]
SL 3770	smooth	100	+	3.0	+	3.5×10^{10}
SA 26	Re	100	+	0.40	+	6.1×10^{9}
SA 33	Re	40	+	0.20	+	2.0×10^{10}
SA 34	Re	100	+	0.60	+	5.9×10^{11}
SA 575	Re	0	-	0	-	1.7×10^{3}
SA 1377	Re	0	-	0	-	1.5×10^{10}

[a] As determined in Figure 9.

[b] As described in Table 2.

[c] As for Table 3, except that only Chi agar plates were used.

[d] As for Table 3, except that Chi phage was titred on lawns of SA 1355.

Table 6. P22 phage mediated transduction of SA1377 genes.[a]

Recombinant classes[b]	Experiment 1	2	3	4	5	Total in each class[c]
Smooth Mot$^+$	22	29	8	15	21	95 (73%)
Smooth Mot$^-$	0	0	0	0	0	0 (0%)
Rough Mot$^+$	17	9	1	3	3	33 (25%)
Rough Mot$^-$	0	1	0	0	1	2 (2%)
Total in each experiment	39	39	9	18	25	130

[a] P22 was induced from SA 1377 by UV induction (10 sec), cleared by centrifugation as in Materials and Methods, chloroform treated, and stored at 4°C. Phage thus obtained was used to transduce SA 2443 by the drop-on-lawn method described in Materials and Methods, with selection for *cysE*$^+$ on MG agar. LPS chemotype and motility were tested as unselected markers.

[b] All recombinants were *cysE*$^+$. LPS type was tested by spotting phages FO, Ffm and P221 (about 10^8 PFU/ml) on recombinants streaked to L-agar plates from 4 h broth cultures. Plates were observed after incubation for 4 h at 37°C. Only the general LPS type could be determined in this way. Motility was tested by stabbing cells from a single colony isolate of the recombinant to a tube of TTC motility agar and incubating for 24 h at 30°C.

[c] These numbers were used to generate the percentages found in Figure 11a.

Table 7. Phage mediated transfer of genes from SL 3748 into SA 2436.[a]

Recombinant classes[b]	Experiment 1	2	3	4	5	6	Total in each class[c]
Smooth Mot$^+$	32	23	33	23	16	22	149 (65%)
Smooth Mot$^-$	0	0	0	1	0	0	1 (0.5%)
Rough Mot$^+$	8	10	1	14	8	8	49 (21%)
Rough Mot$^-$	10	9	6	2	3	2	32 (14%)
Total in each experiment	50	42	40	40	27	32	231

a Phages ES18.h1 or 19.h1 were prepared by growth on SL 3748 as described in the Materials and Methods. Lysates of these phages were then used to transduce SA 2436, selecting for prototrophy by growth on MG agar.

b All recombinants were *pyrE*$^+$. LPS type and motility were tested as described in Table 6.

c These totals were used to generate the percentages found in Figure 12.

Table 8. Phage mediated transduction of genes from SL 3748 into *cysE*$^-$ strains.[a]

Recombinant classes[b]	Experiment 1	2	3	4	5	6	7	8	Total in each class[d]
Smooth Mot$^+$	30	31	30	30	11	19	32	30	213 (79%)
Smooth Mot$^-$	3	2	0	0	0	0	0	2	7 (3%)
Rough Mot$^+$	0	0	1	9	1	2	6	3	22 (8%)
Rough Mot$^-$	7	6	9	1	5	2	2	5	29 (11%)
Total in each experiment	40	39	32	40	17	23	40	40	271

[a] Phages ES18.h1 of 19.h1 prepared on SL 3748 were used to transduce SA 2443 or SA 2568. Prototrophy was selected for by growth on MG agar. Results were similar for all combinations of recipient and transducing phage used.

[b] All recombinants were *cysE*$^+$. LPS type and motility were tested as in Table 6.

[c] Five additional experiments resulted in only Smooth Mot$^+$ (178 transductants) and Rough Mot$^-$ (30 transductants) classes. These results were not used for calculating totals in this table or for the calculations of percentages in Figure 14.

[d] These totals were used to calculate the percentages found in Figure 13.

Table 9. Motility of strains involved in transductions of mot-401.

Strain	LPS type[a]	Source[b]	Motility in TTC tubes (30°C)[c]	% cells motile in microscope[c]	Motility on TTC agar plates: rate of movement (mm/h)[c]	Average number of flagella/cell[d]
SA 2436	smooth	(recipient)	+	100	2.76	16
SA 2443	smooth	(recipient)	+	50	2.24	10
SA 2568	smooth	(recipient)	+	100	2.52	3.9
SA 2963	smooth	SL 3748 x SA 2436	+	80	2.44	ND[e]
SA 2974	rough	SL 3748 x SA 2436	+	40	0.13[f]	3.5
SA 2939	smooth	SL 3748 x SA 2443	-	0	0	13
SA 2940	smooth	SA 2939 x SA 2443	-	0	0	13
SA 2971	rough	SL 3748 x SA 2443	-	0	0	3.0

[a] Determined by phage sensitivity. Since SDS-PAGE of prepared LPS was not performed for transductants, no specific chemotype was assumed for the LPS. The full genotype of each strain is listed in Table 1.

[b] The transductions giving rise to the strain are noted. The source of recipient strains is given in Table 1.

[c] As in Table 2. [d] As calculated in Fig. 8. [e] ND means not determined.

[f] This strain showed a "Sat" (sattelite colony) phenotype. See Fig. 6a and the Discussion for explanation.

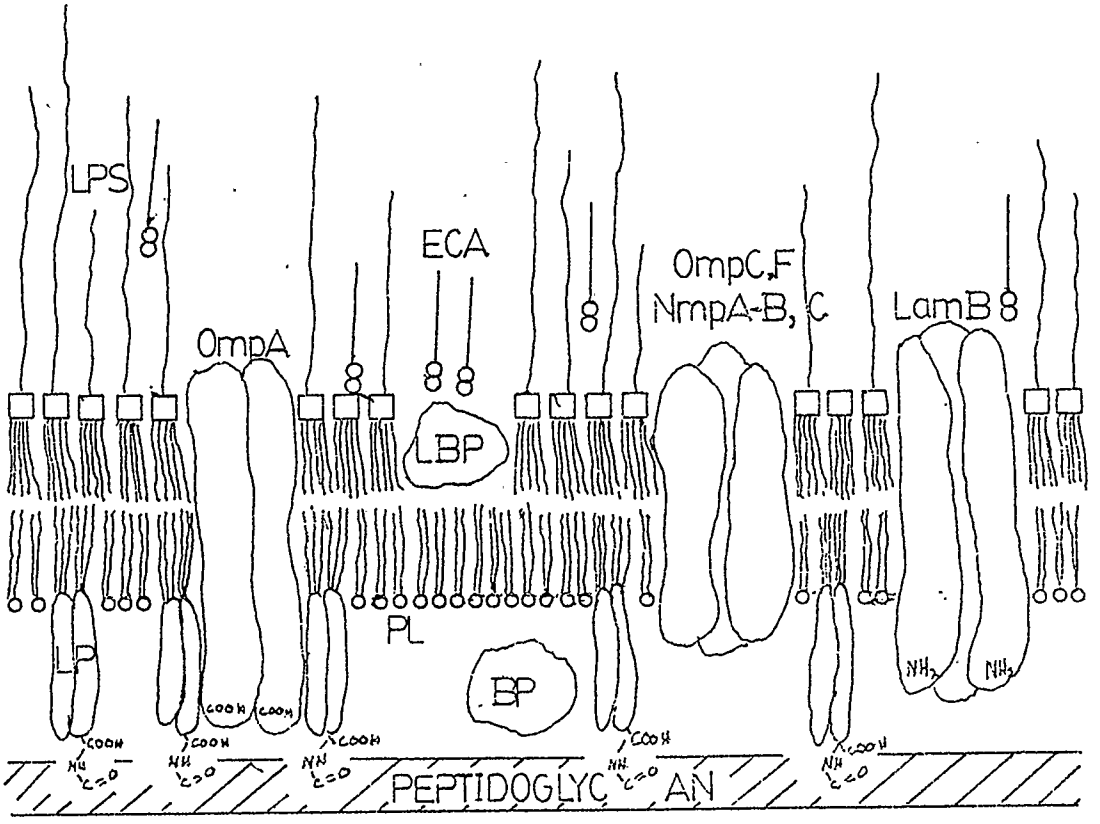


Figure 1. A schematic representation of the OM of Gram-negative bacteria. (Adapted from Osborn and Wu, 1980)

LPS	lipopolysaccharide
PL	phospholipid
ECA	enterobacterial common antigen
LBP	LPS binding protein
BP	periplasmic binding protein
OmpA	outer membrane protein A
OmpC,F, LamB, NmpA-B,C	outer membrane proteins
LP	lipoprotein

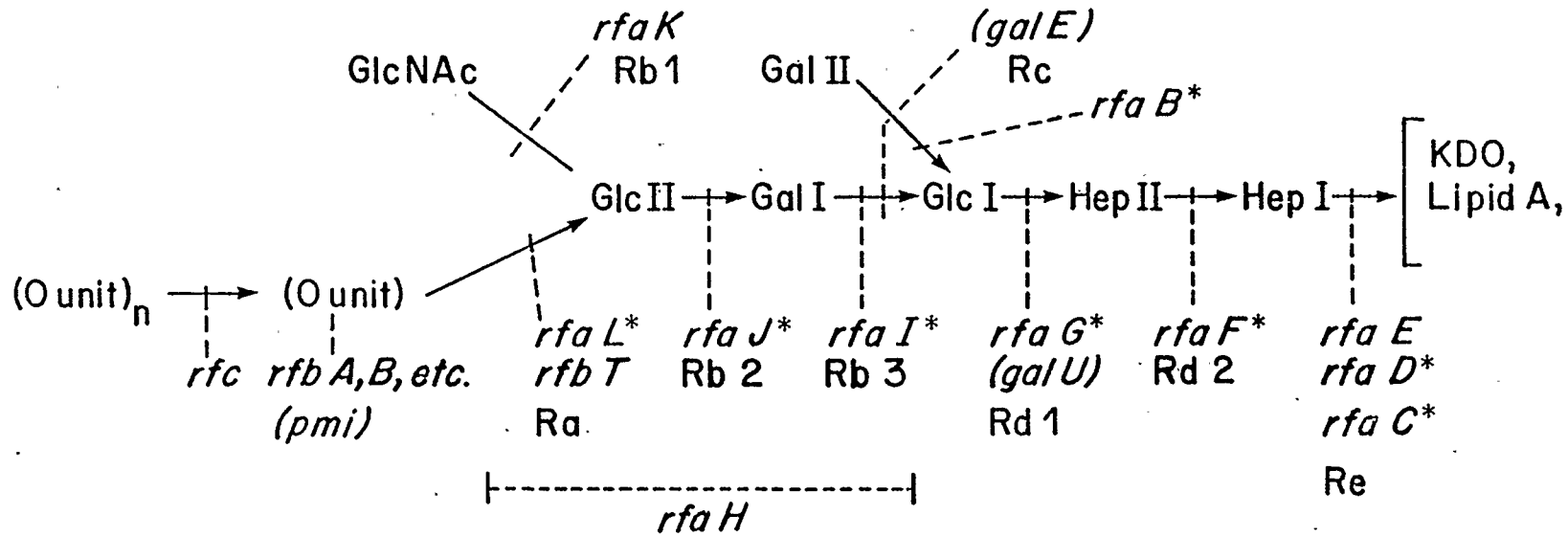


Figure 2. A schematic representation of the LPS of Salmonella typhimurium. The italicized letters are genes responsible for the production of glycosyltransferases which make the core and O-antigen of the LPS; dotted lines indicate the block in synthesis resulting from mutations in each of these genes. Below the genotypes, the LPS chemotype is given in block letters.

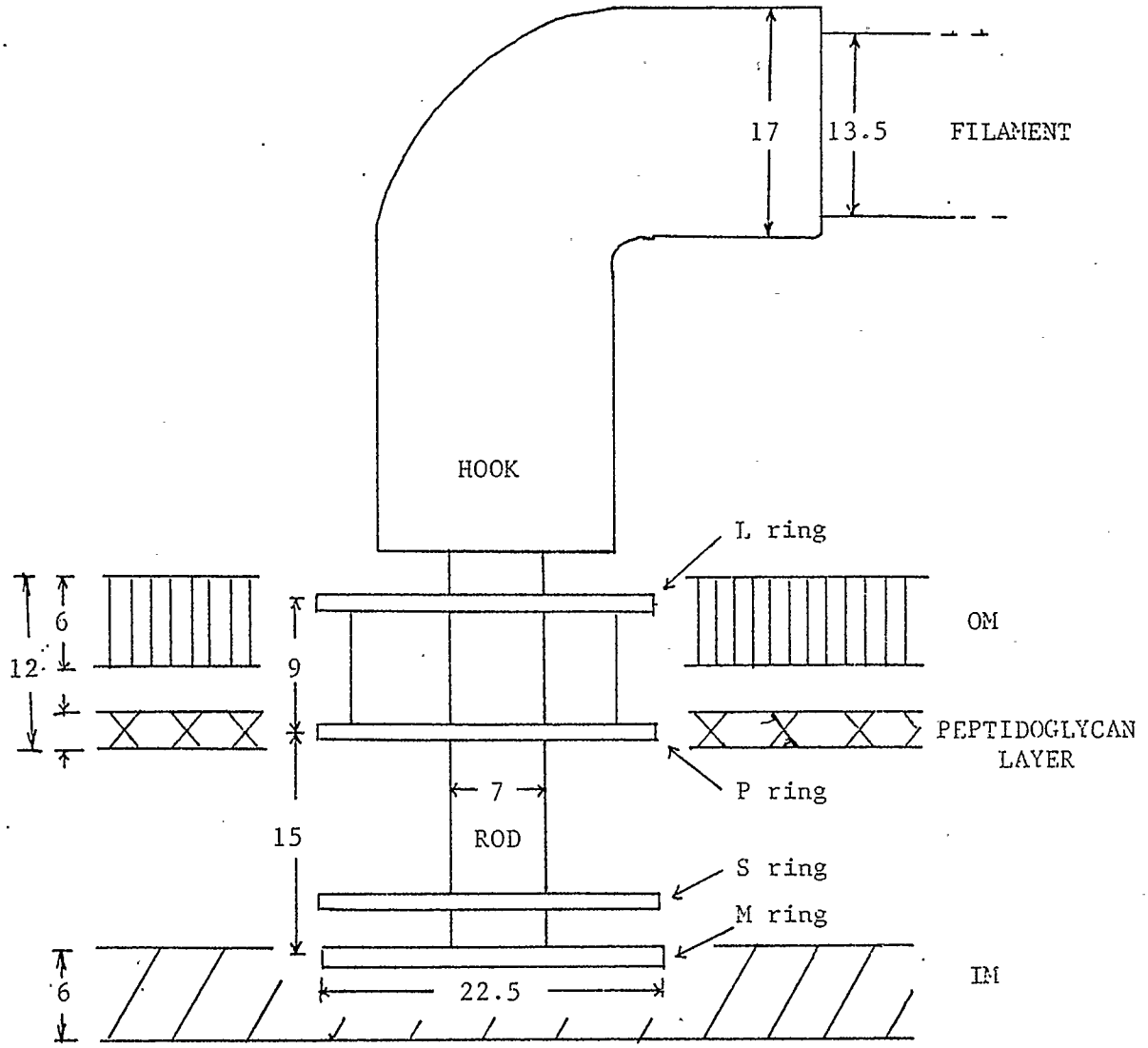


Figure 3. Model of the basal body of a flagellum from a Gram-negative bacterium showing the attachment of the basal body to the cell envelope. All dimensions are in nm. (Adapted from DePamphilis and Adler, 1971c)

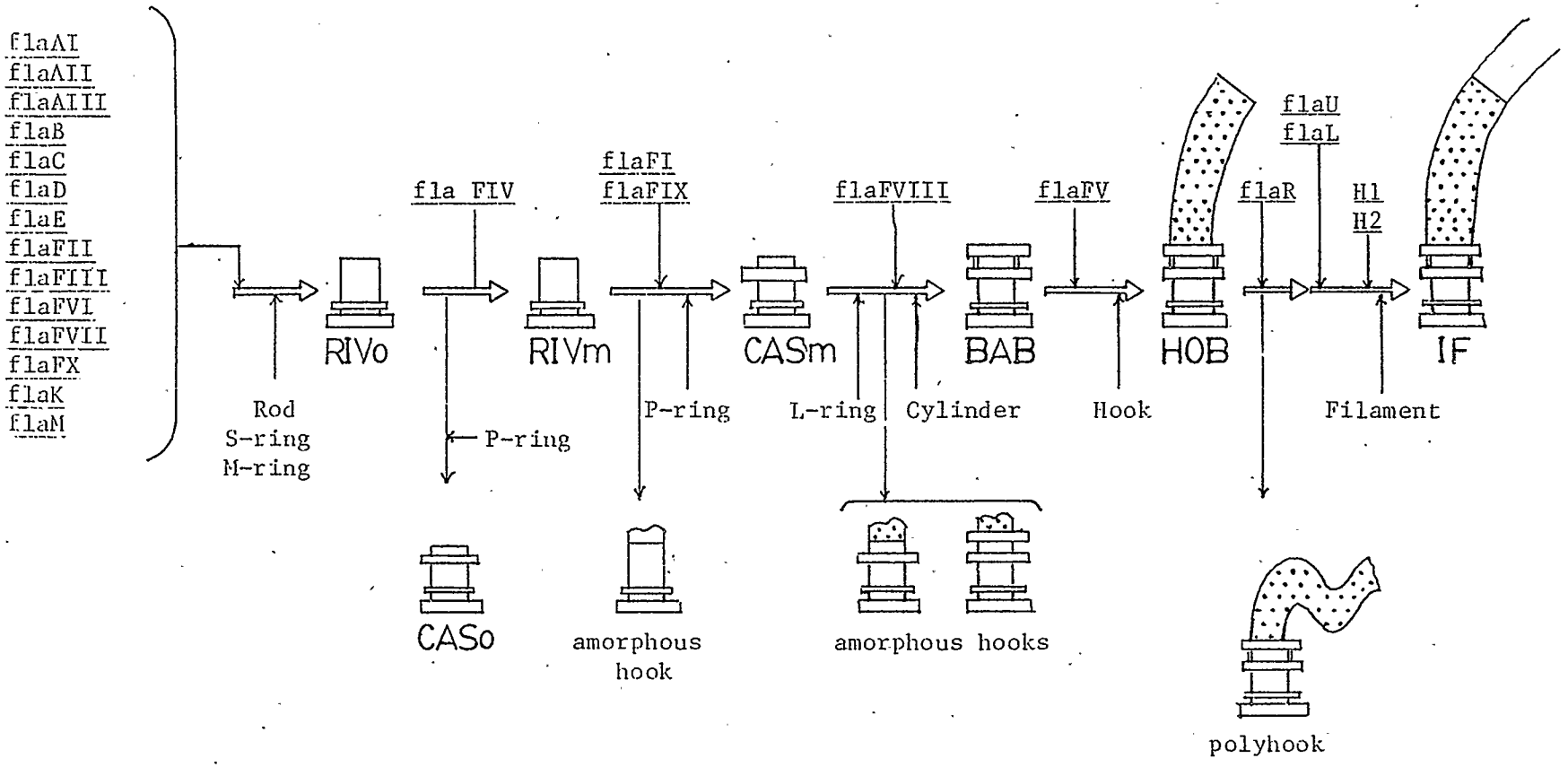


Figure 4. The process of flagellar morphogenesis in Salmonella. RIVo and RIVm: rod-inner ring complexes; CASo and CASm: RIV-P ring complexes: BAB basal body; HOB: hook-basal body complex; IF: intact flagellum. (Adapted from Iino, 1978)

Figure 5. Linkage map of Salmonella typhimurium showing the genes responsible for LPS biosynthesis (rfa and rfb), flagellar biosynthesis (fla), motility (mot) and chemotaxis (che). The rfb locus contains several rfb genes which are not individually shown; as well, genes responsible for the synthesis of Lipid A are not shown.

(adapted from Sanderson and Roth, 1984)

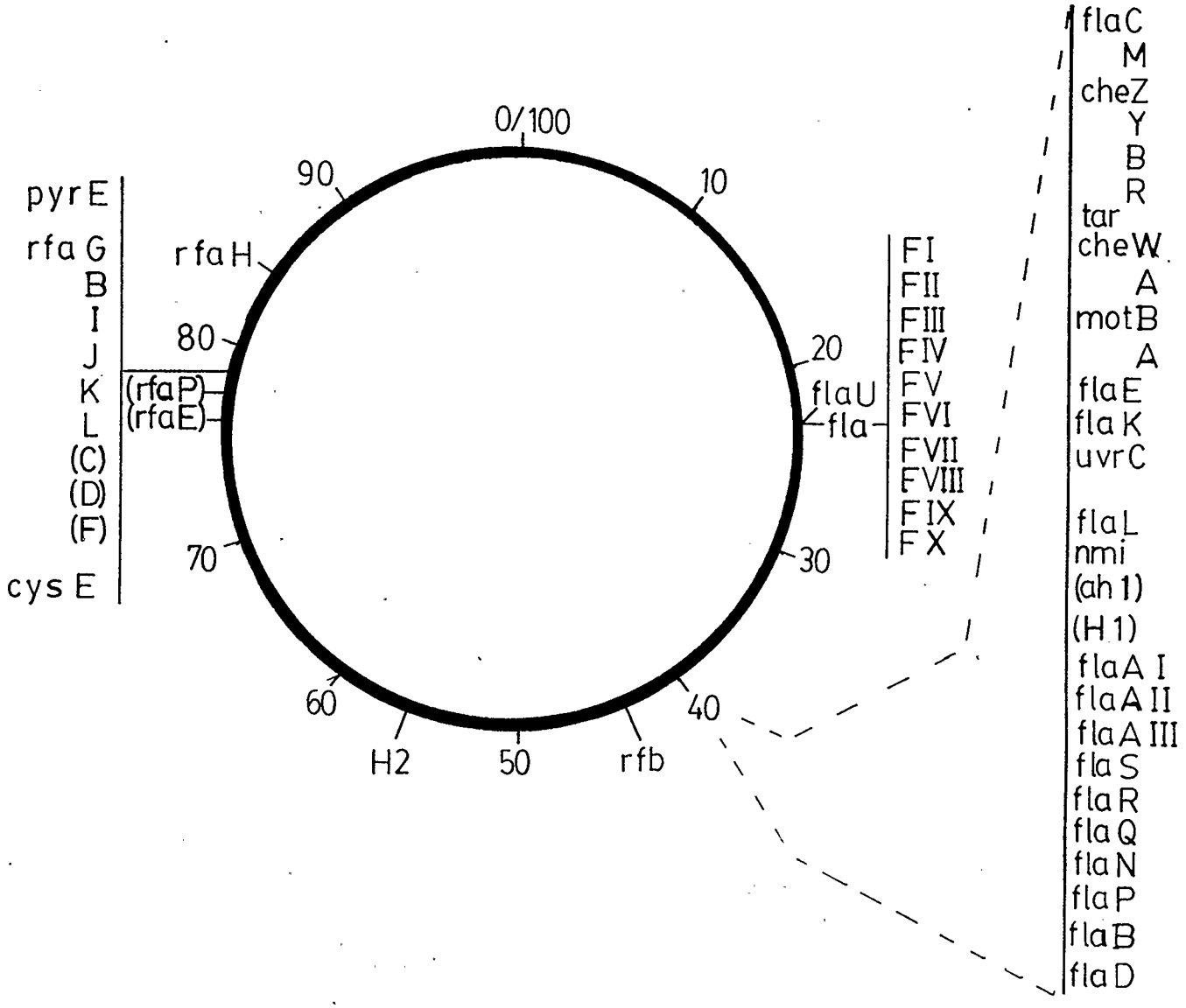
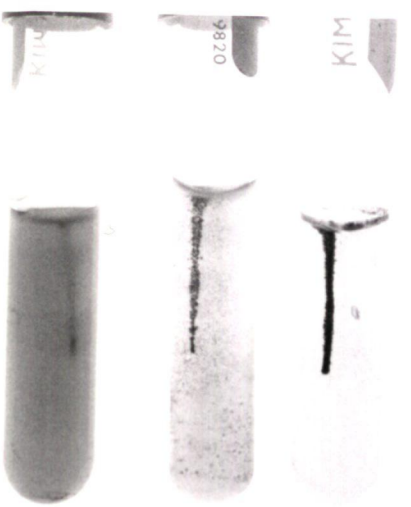

0/100
10
20
30
40
50
rfb
H2
60
70
80
90
pyrE
rfa G
B
I
J
K
L
(C)
(D)
(F)
cys E
rfaH
(rfaP)
(rfaE)
flaU
fla
FI
FII
FIII
FIV
FV
FVI
FVII
FVIII
FIX
FX
flaC
M
cheZ
Y
B
R
tar
cheW
A
motB
A
flaE
flaK
uvrC
flaL
nmi
(ah1)
(H1)
flaA I
flaA II
flaA III
flaS
flaR
flaQ
flaN
flaP
flaB
flaD

Figure 6. Motility of bacteria in TTC motility medium.

a. Bacteria were stabbed to TTC motility medium in tubes and incubated for 24 hours at 30 degrees C. Tube 1 contains SL 3770, which is fully motile; tube 2 contains SL 3749; and tube 3 contains SL 3748, which is Mot^-. SL 3749 exhibits a type of motility in which colonies form at some distance from the original, discrete stab. I have called these colonies satellite colonies and the motility satelliting (Sat).

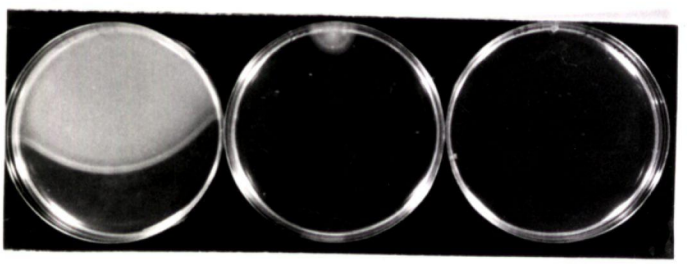
b. Bacteria were stabbed to TTC motility medium in plates and were incubated at 30 degrees C for 24 hours. Plate 1 was inoculated with SL 3770 (rfa$^+$ mot$^+$), plate 2 with SL 3750 (rfaJ417), and plate 3 was inoculated with SL 3748 (rfaI432 mot-401). Areas of growth show as light regions which are turbid and, on the original plates, red in colour from the reduction of TTC by growing bacteria.

a



1 2 3

b



1 2 3

Figure 7. EM observations of flagella on Salmonella typhimurium.

a. Fla^- cells of the strain SA 1649

b. Fla^+ cells of the strain SA 2436

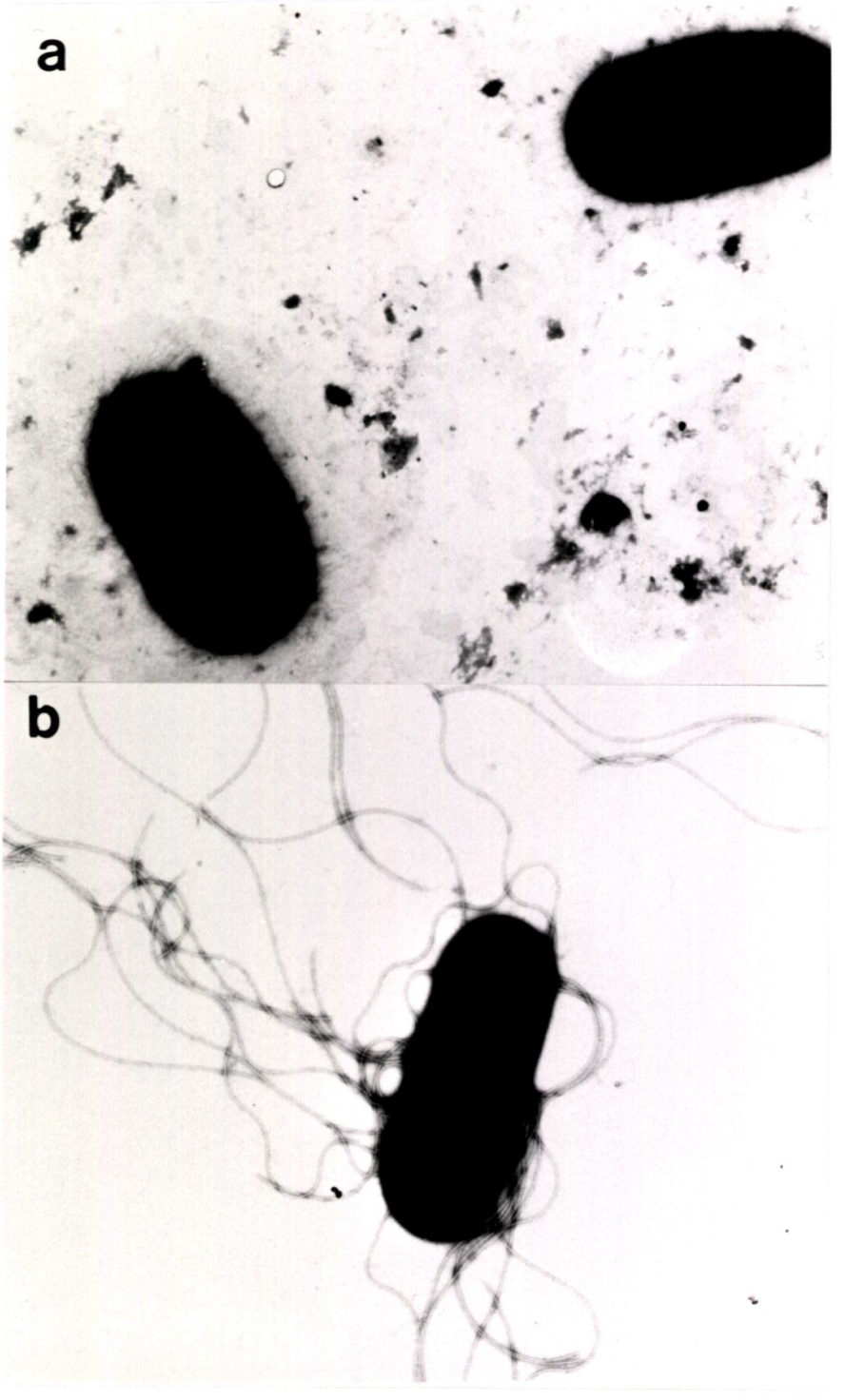
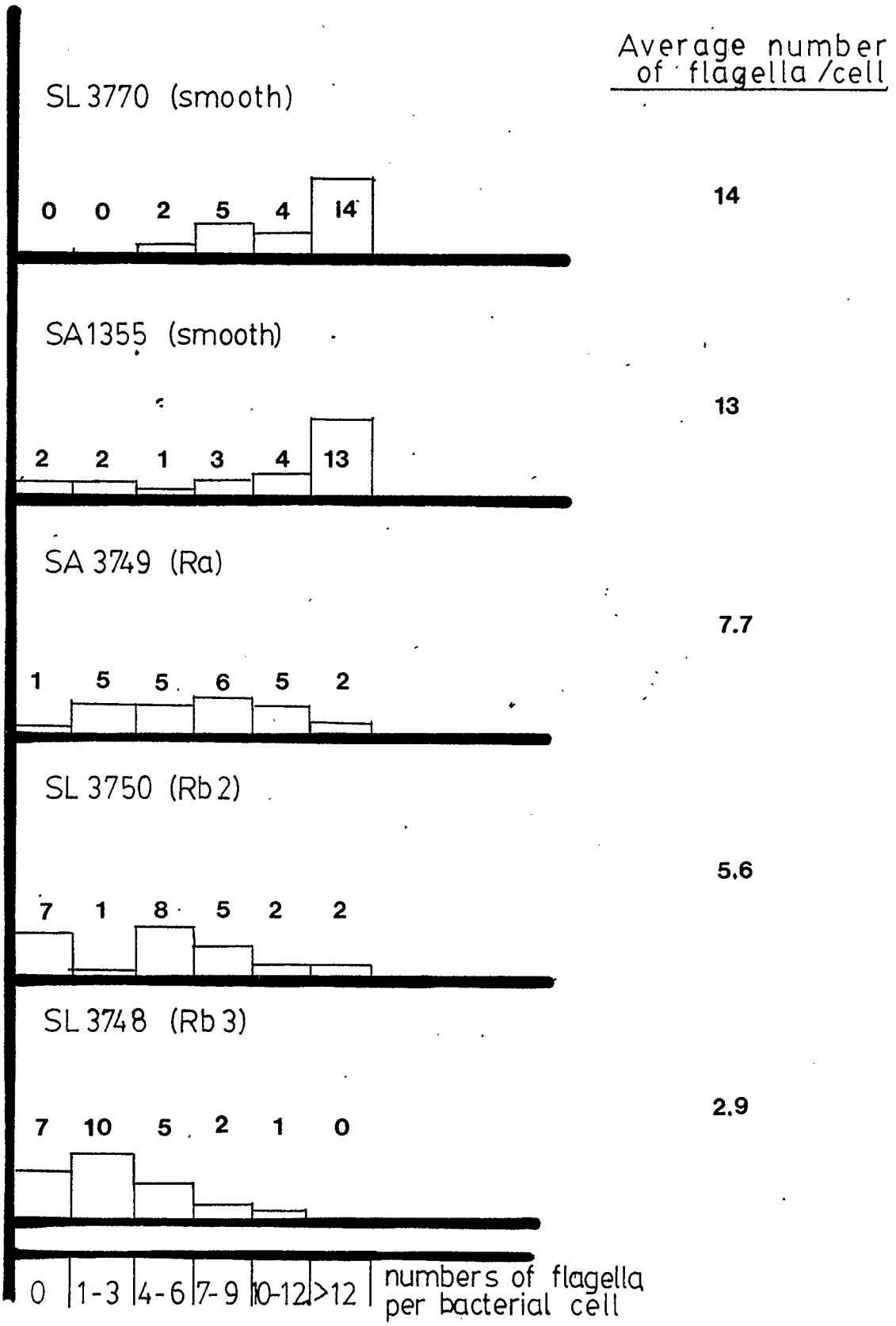

a
b

Figure 8. Flagella of S. typhimurium standard smooth and rough strains.

The numbers of flagella on each cell were counted for 25 negatively-stained cells of each strain. The first 25 individual cells encountered were used for counting flagella; bacteria which were in clumps were not included. Cells were assigned to arbitrarily determined classes consisting of bacteria with 0, 1 to 3, 4 to 6, 7 to 9, 10 to 12, and greater than 12 flagella per cell.

Histogram bars were drawn to represent the number of bacteria in each of the above classes. The height of the bar is proportional to the number of bacteria belonging to each class and the number above each bar is the actual number of bacteria in that class. The average number of flagella per cell is a number derived by multiplying the number of bacteria in each class by the median numbers of flagella per cell for that class. The results for each class were added together, then divided by the total number of bacteria counted per strain (25). An estimate of 18 flagella per cell was used as the median value for the class of bacteria having greater than 12 flagella per cell. This number was based on a sample count of bacteria in this class (data not shown).


Average number of flagella /cell
SL 3770 (smooth)
0 0 2 5 4 14
14
SA1355 (smooth)
2 2 1 3 4 13
13
SA 3749 (Ra)
1 5 5 6 5 2
7.7
SL 3750 (Rb 2)
7 1 8 5 2 2
5.6
SL 3748 (Rb 3)
7 10 5 2 1 0
2.9
0 1-3 4-6 7-9 10-12 >12
numbers of flagella per bacterial cell

(continued)

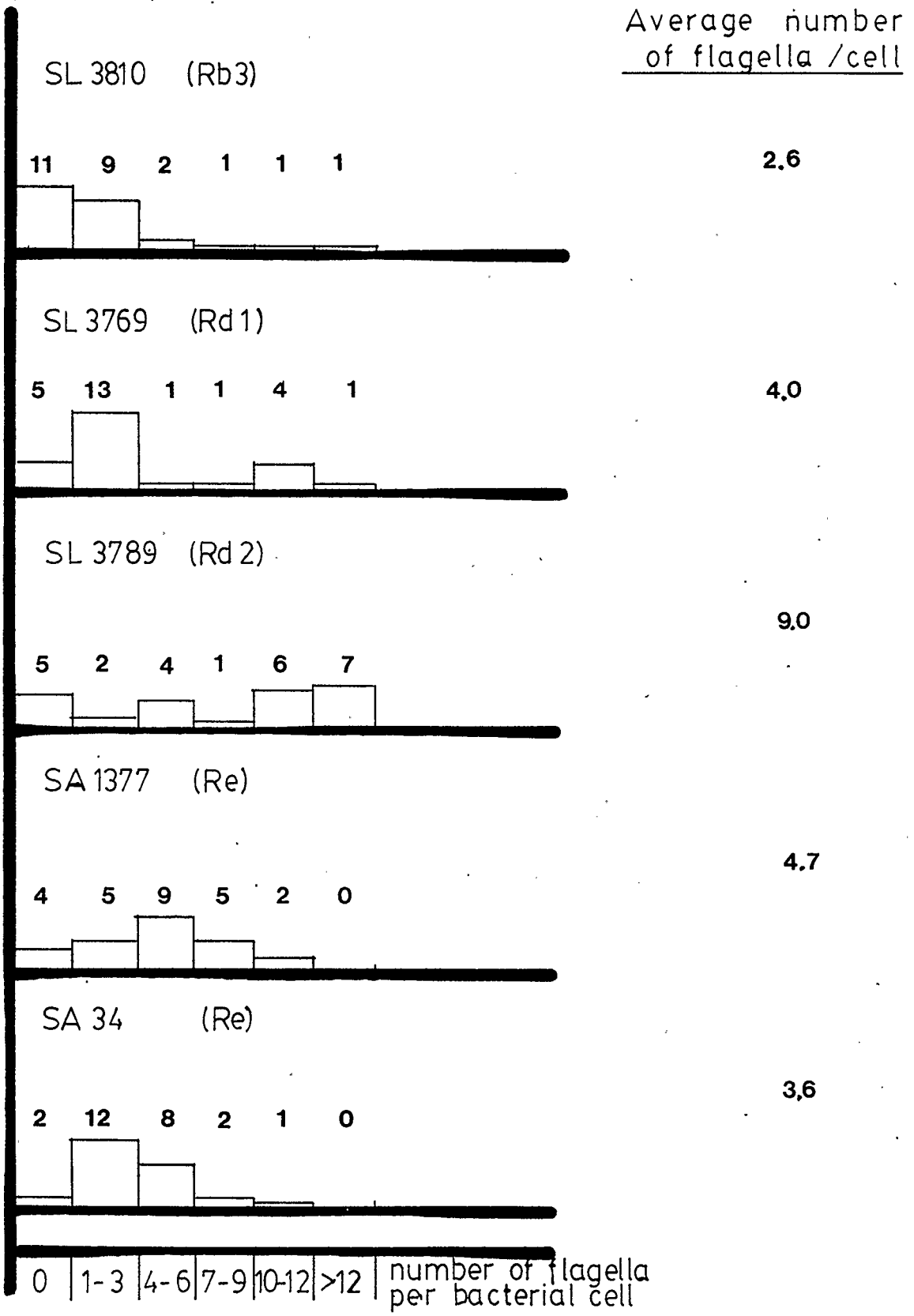

Average number of flagella /cell
SL 3810 (Rb3)
11 9 2 1 1 1
2.6
SL 3769 (Rd 1)
5 13 1 1 4 1
4.0
SL 3789 (Rd 2)
9.0
5 2 4 1 6 7
SA 1377 (Re)
4.7
4 5 9 5 2 0
SA 34 (Re)
3.6
2 12 8 2 1 0
0 | 1-3 | 4-6 | 7-9 | 10-12 | >12 | number of flagella per bacterial cell

Figure 9. SDS-PAGE of LPS from various strains, silver stained by the method of Hitchcock and Brown (1983).

Lanes 1 through 6 are standard strains. A partial genotype of each strain is given in parentheses, and after that the LPS chemotype is given.

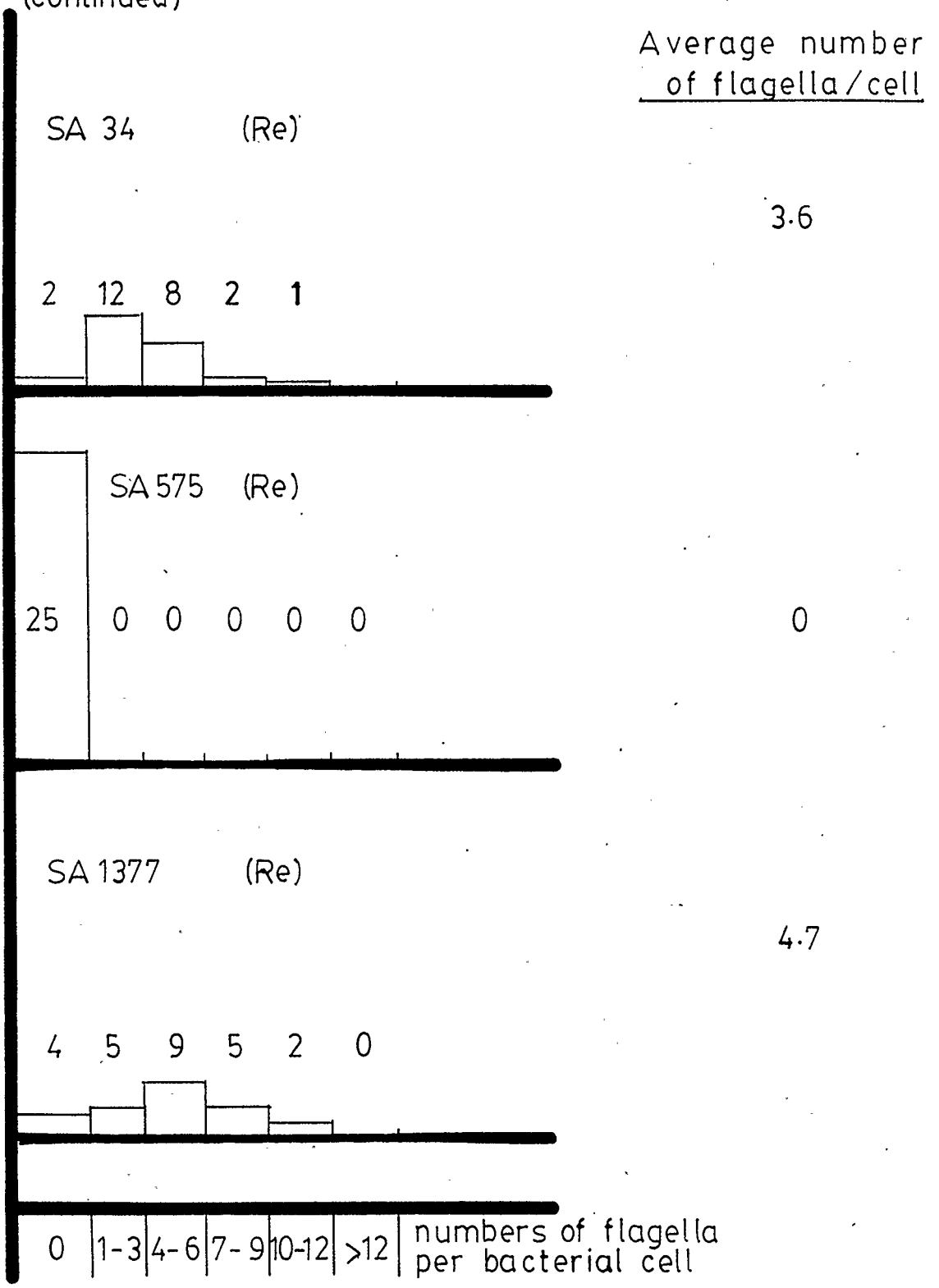
lane 1	SL 3770	(*rfa*$^+$)	smooth
lane 2	SL 3750	(*rfaJ 417*)	Rb2
lane 3	SL 3748	(*rfaI 432*)	Rb3
lane 4	SL 3769	(*rfaG 471*)	Rd1
lane 5	SL 3789	(*rfaF 511*)	Rd2
lane 6	SA 1377	(*rfaC 630*)	Re
lane 7	SA 22	(*rfa*$^+$)	smooth
lane 8	SA 26	(*rfaE 629*)	Re
lane 9	SA 33	(*rfaC 630*)	Re
lane 10	SA 34	(*rfa-631*)	Re
lane 11	SA 35	(*rfa-632*)	Rb3
lane 12	SA 575	(*rfa-543*)	Re
lane 13	SA 1648	(*rfa*$^-$)	Ra
lane 14	SA 1649	(*rfaE 543*)	Re


1 2 3 4 5 6 7 8 9 10 11 12 13 14

Figure 10. Flagella of Re chemotype strains.

Flagellar numbers were determined as described for Figure 8. Histograms were also constructed in the same manner.

(continued)


Average number of flagella/cell
SA 34 (Re)
3.6
2 12 8 2 1
SA 575 (Re)
25 0 0 0 0 0
0
SA 1377 (Re)
4.7
4 5 9 5 2 0
0 1-3 4-6 7-9 10-12 >12
numbers of flagella per bacterial cell

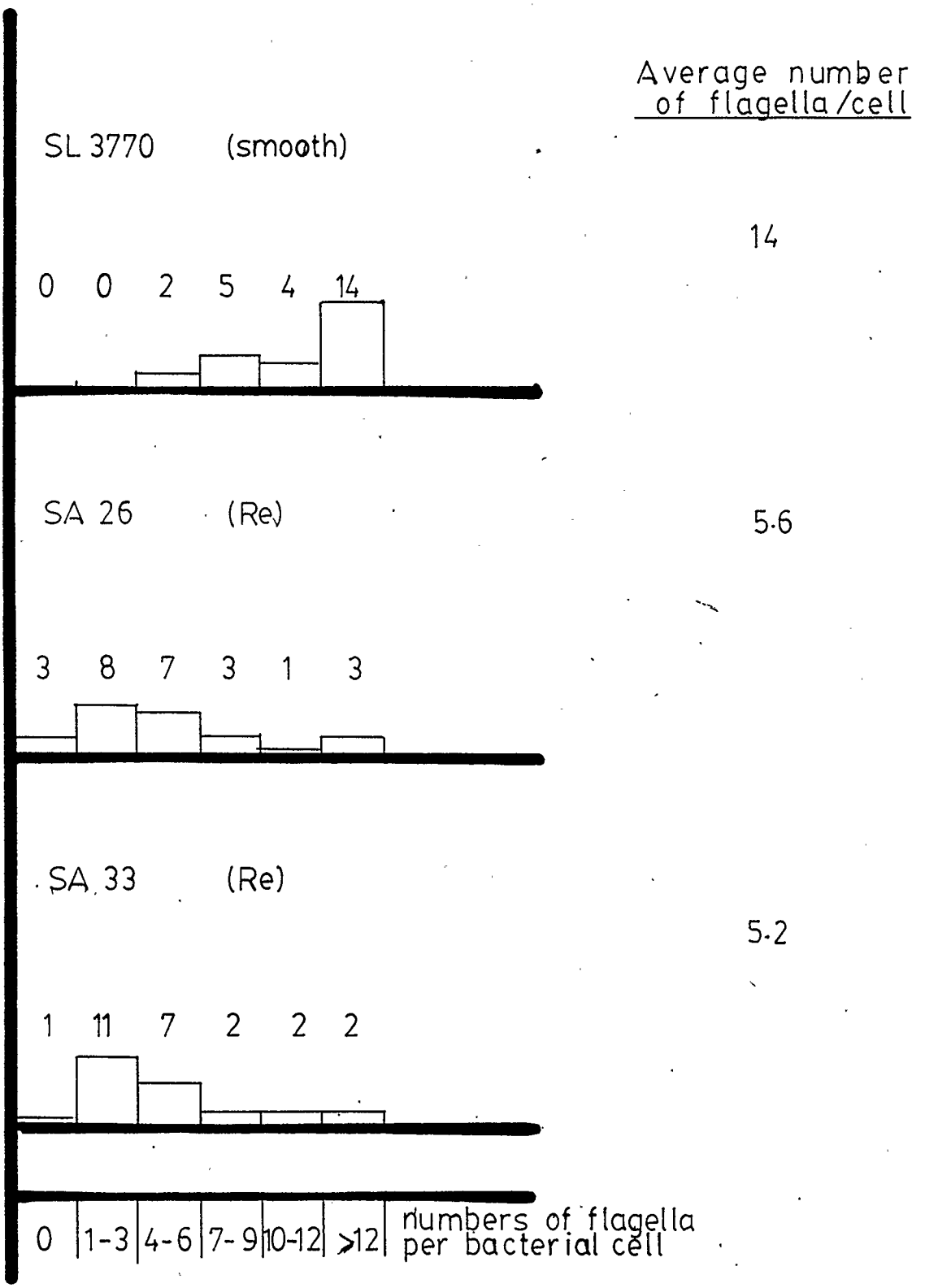

Average number of flagella/cell
SL 3770 (smooth)
14
0 0 2 5 4 14
SA 26 (Re)
5·6
3 8 7 3 1 3
SA 33 (Re)
5·2
1 11 7 2 2 2
0 1-3 4-6 7-9 10-12 >12
numbers of flagella per bacterial cell

Figure 11. Crossover diagrams for the transduction of genes from SA 1377 into auxotrophic strains.

a. Transductions into SA 2443 (cysE 396). The transductions were performed as in the footnotes to Table 6, with each recombinant class being represented as a percentage of total recombinants obtained. (See Table 6)

b. Transductions into SA 2436 (pyrE 123). Data was obtained from a single experiment which is described in the text. Recombinants in each class are expressed as a percentage of total recombinants obtained.

In both diagrams, patterns of recombination were arranged to minimize the number of double crossover events.

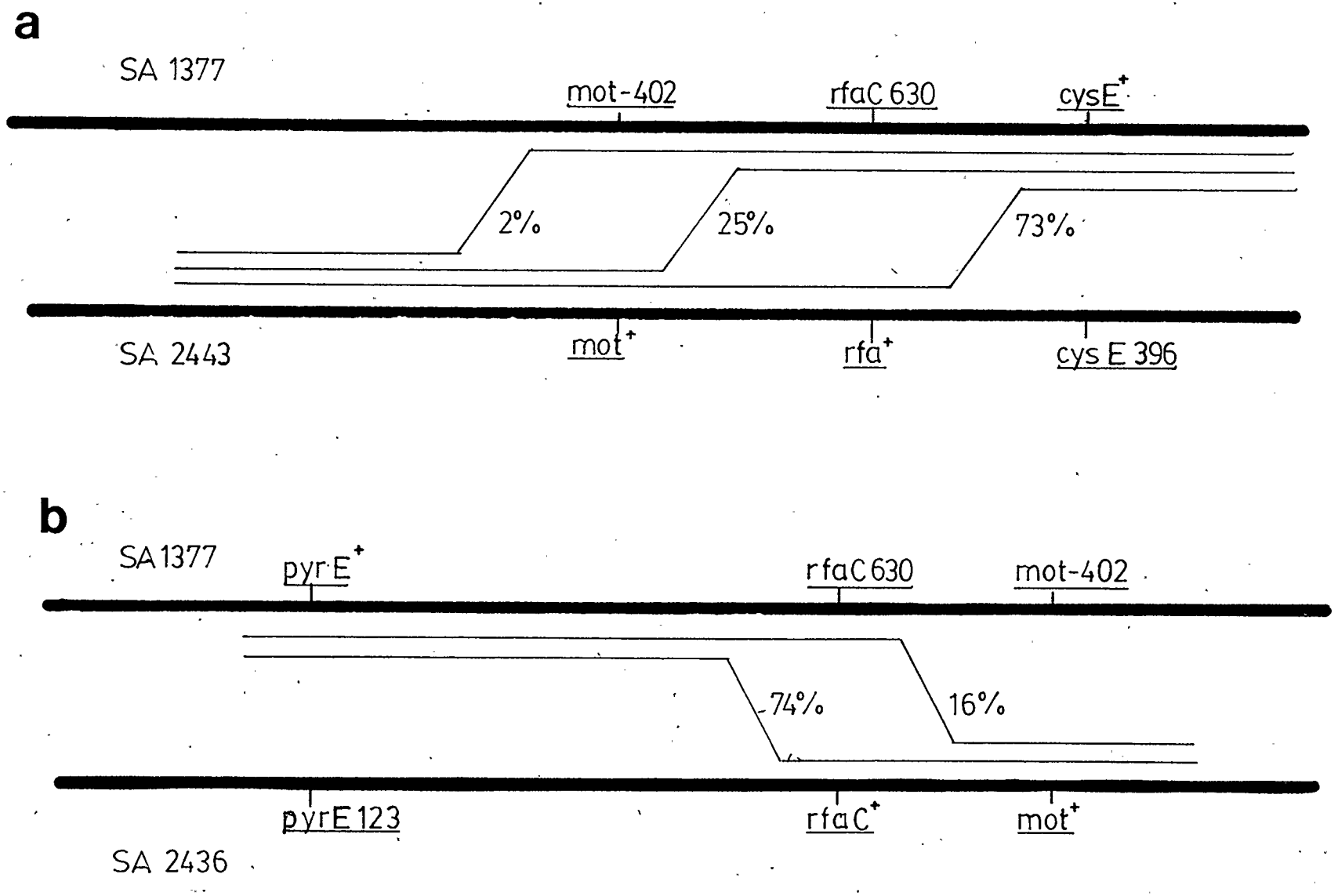

a
SA 1377
mot-402
rfaC 630
cysE+
2%
25%
73%
SA 2443
mot+
rfa+
cys E 396
b
SA1377
pyr E+
rfaC630
mot-402
74%
16%
pyrE 123
rfaC+
mot+
SA 2436

Figure 12. Crossover diagrams for the transductions of genes from SL 3748 into SA 2436.

Transductions were performed as specified in Table 7. Each recombinant class is expressed as a percentage of total transductants obtained.

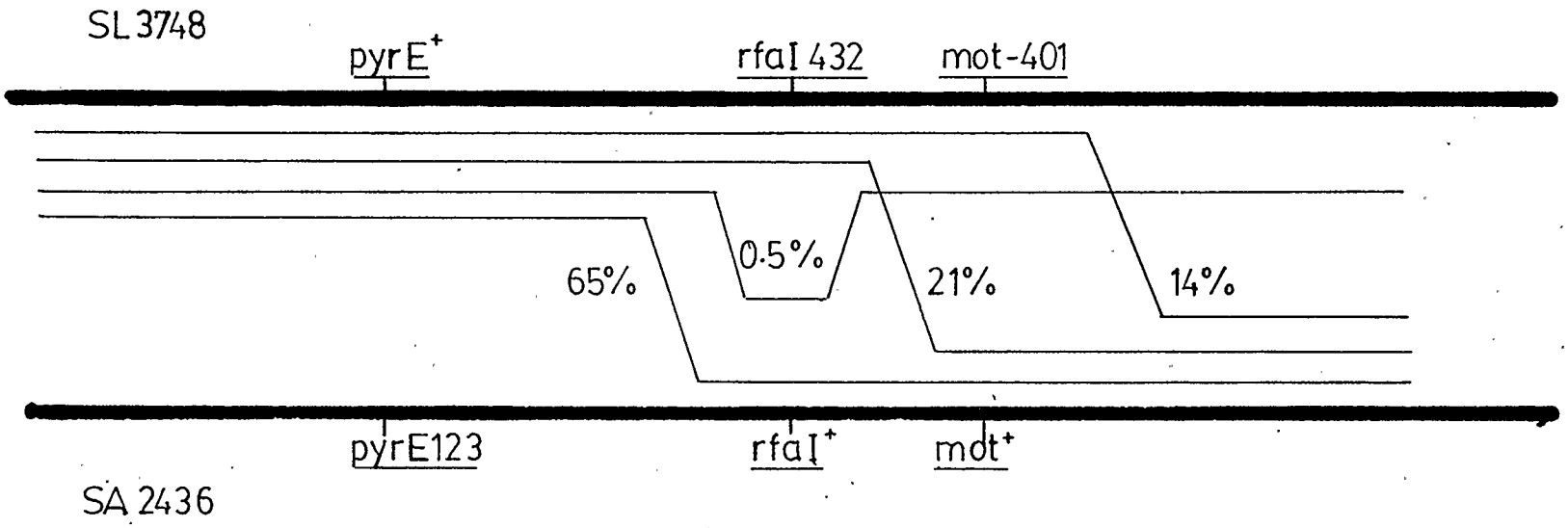

SL 3748
pyrE+
rfaI 432
mot-401
65%
0·5%
21%
14%
pyrE123
rfaI+
mdt+
SA 2436

Figure 13. Crossover diagrams for the transduction of genes from SL 3748 into cysE recipients.

Transductions were performed as described in Table 8. The frequencies of co-transduction obtained allow the crossover diagrams to be drawn in two ways, depending on which event is taken to represent the double-crossover event. Diagram 'a' assumes that mot-401 is between rfaI 432 and cysE, while in 'b' mot-401 has been placed between pyrE and rfaI 432.

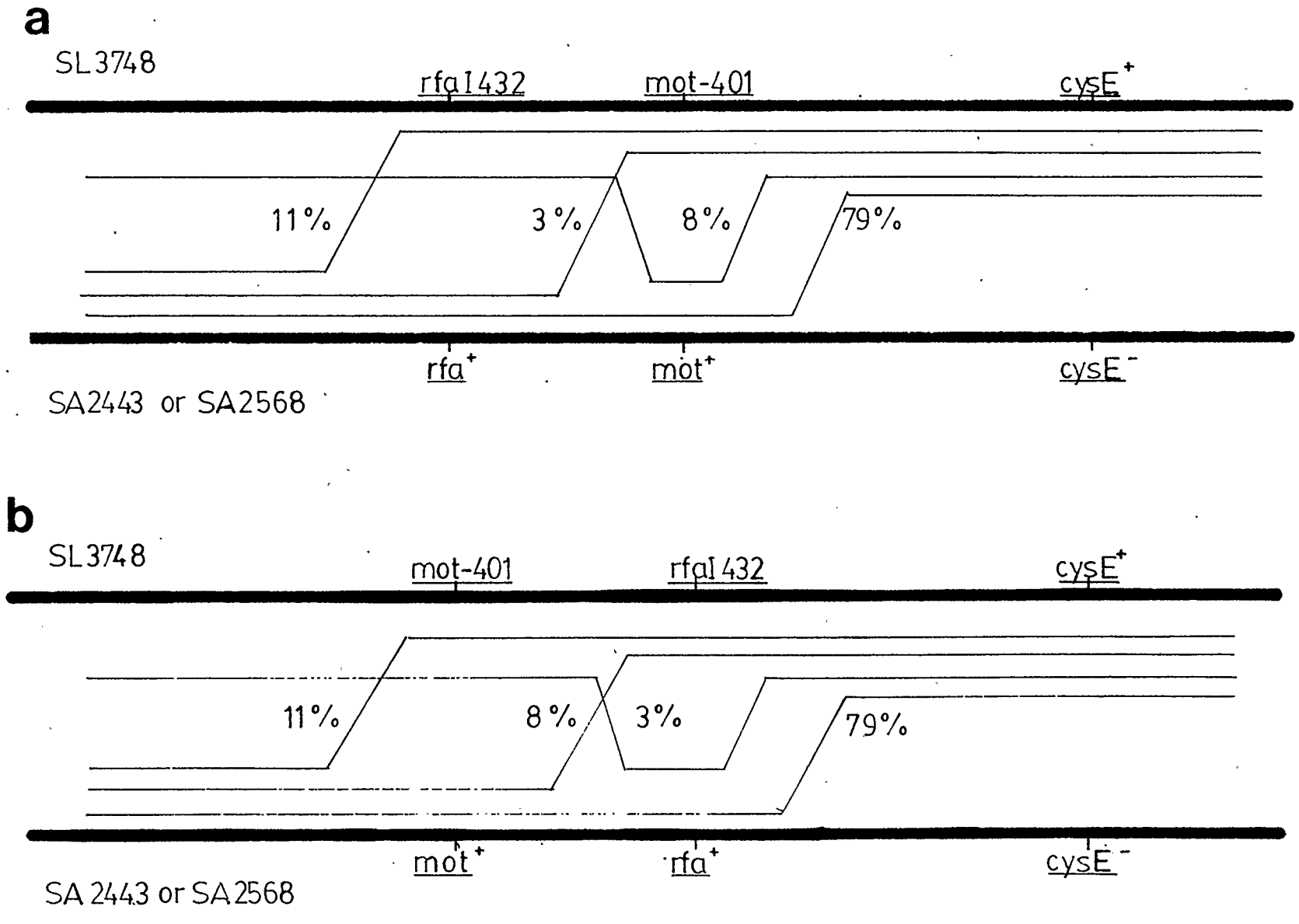

a
SL3748
rfa1432
mot-401
cysE+
11%
3%
8%
79%
rfa+
mot+
cysE-
SA2443 or SA2568
b
SL3748
mot-401
rfa1432
cysE+
11%
8%
3%
79%
mot+
rfa+
cysE-
SA 2443 or SA2568

Figure 14. Plasmids of strains resulting from the conjugation of pKZ3 into SA 2954.

The protocol used for conjugation is described in the text. DNA was isolated by the method outlined in Materials and Methods, and was electrophoresed in 0.7% agarose for 26 hours.

Lanes 1 and 20	DNA molecular weight marker I
Lanes 2 through 17	transconjugants
Lane 18	pKZ3
Lane 19	SA 2954

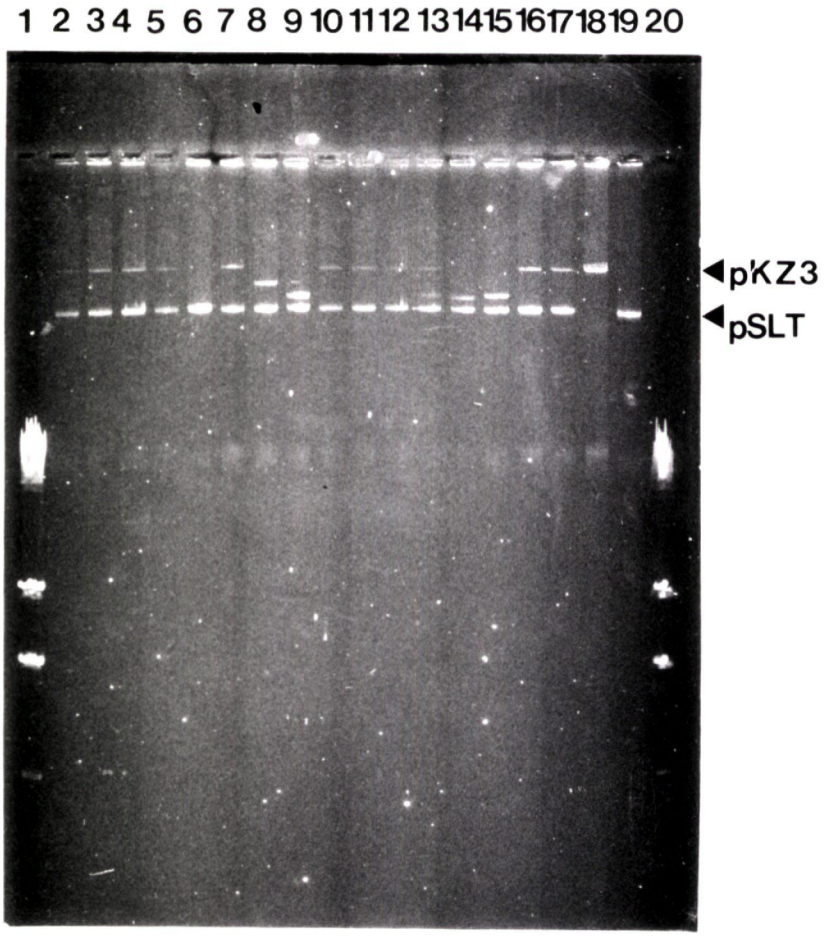

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
pKZ3
pSLT

Figure 15. Gel electrophoresis of plasmid DNA from strains with plasmids carrying the mot-401 gene.

Conjugations were performed as described in the text. The data shown represents a composite from three different experiments. Lanes 1 through 4 are strains obtained from the transfer of pULB 113 from SA 2941 into SA2389; lanes 6, 7, and 11 are strains resulting from the transfer of pULB 113 from SA 2941 into SA 2386; lanes 7 through 10 are strains resulting from the conjugation of DNA from SA 2948 (lane 2) into SA 572.

Lane 1	SA 2941	carries pULB 113 and pSLT
Lane 2	SA 2948	carries pSLT and pKZ35
Lanes 3 & 4	not stocked	carry plasmids similar to pKZ35
Lane 5	SA 2972	carries pSLT and pKZ36 (faint band below pSLT)
Lane 6	SA 2973	carries pSLT; pKZ37 is too faint to see in this lane
Lanes 7 - 10	not stocked	Lanes 7,8, and 9 show pSLT and pULB113; only lane 10 shows pSLT and pKZ35
Lane 11	SA 2973	carries pSLT and pKZ37 (faint band above pSLT)

Lanes from different gels were aligned using pSLT. Strains carrying pSLT alone were included in each gel (data not shown).

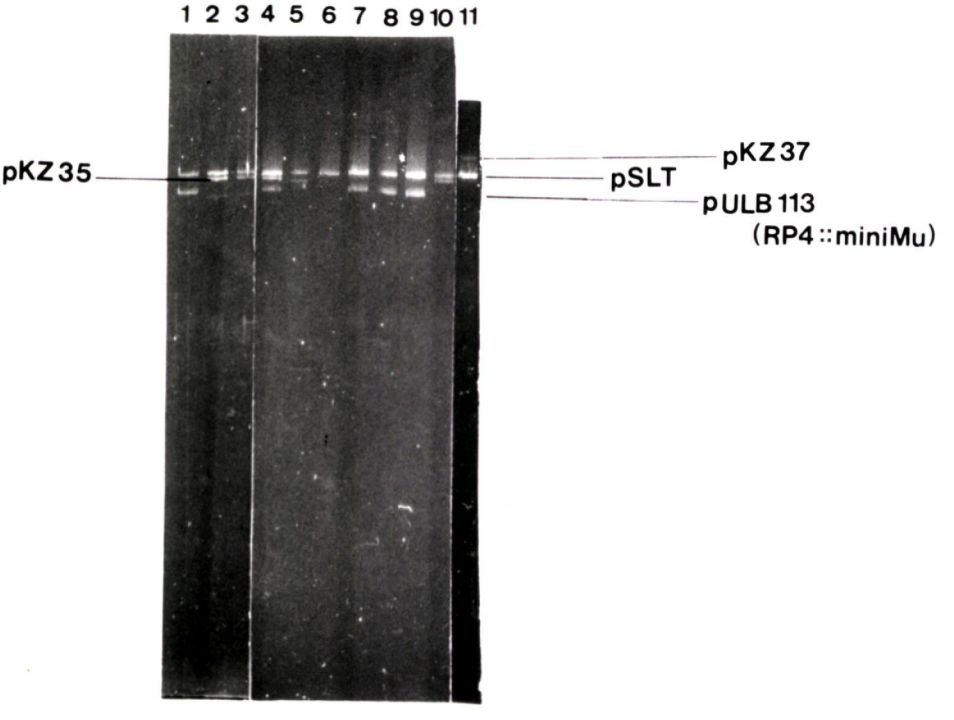

1 2 3 4 5 6 7 8 9 10 11
pKZ35
pKZ37
pSLT
pULB113
(RP4::miniMu)

Figure 16. Reversion of SA 2940 Mot^- cells to Mot^+ when streaked on TTC motility agar.

An ON culture of the organism in L-broth was streaked to TTC motility agar and incubated 48 hours at 30 degrees C. Four areas of swarming, or motile, cells can be seen originating from the stread and extending some distance from the streak. These areas are brighter and appear less dense in the photograph.

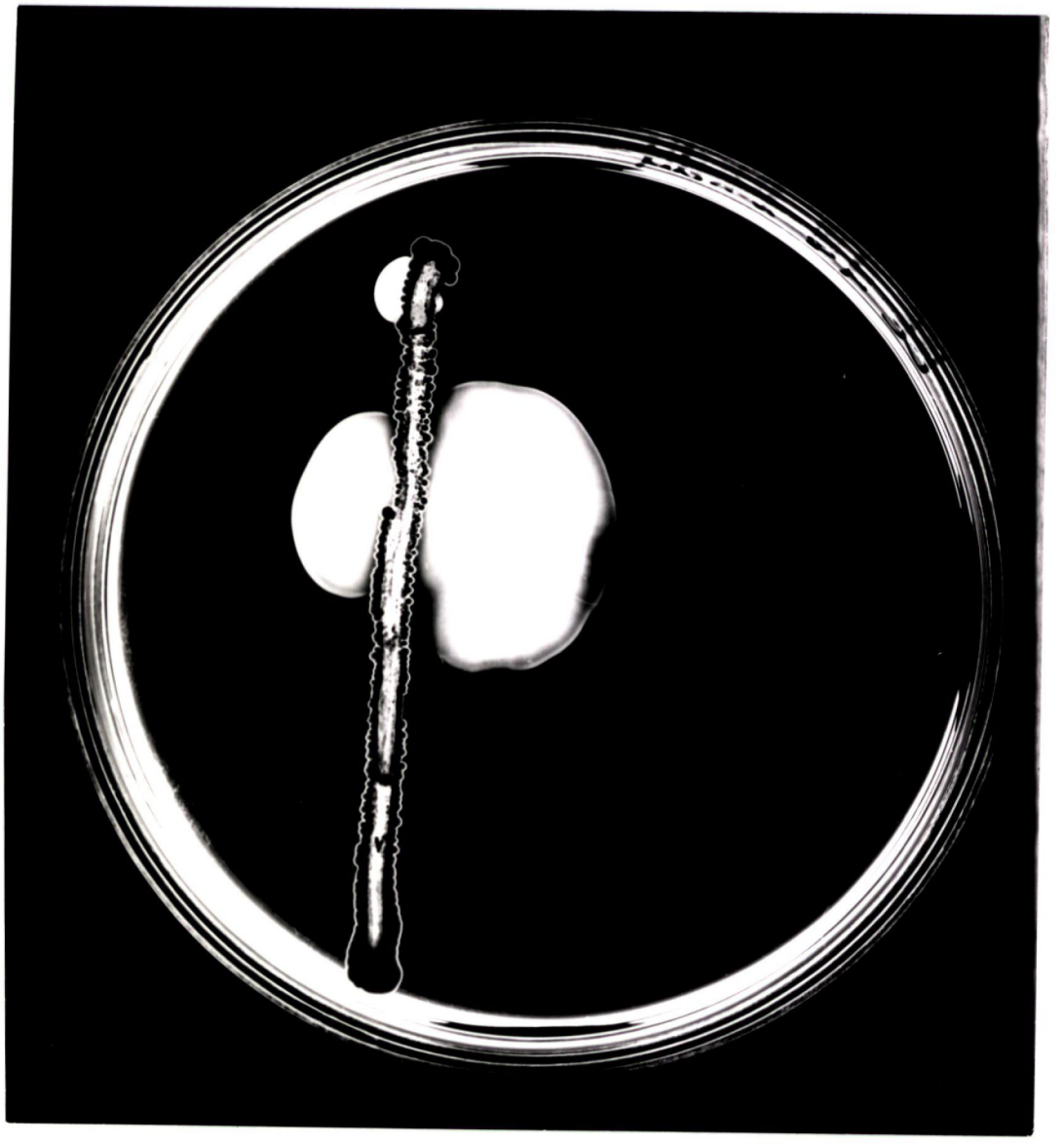


Figure 17. Reversion of SA 2940 from Mot^- to Mot^+ in a pour plate of TTC motility agar.

A 5 hour L-broth culture of the organism was diluted to 10^{-7} and 0.1 ml of this dilution was incorporated into TTC motility agar to produce a pour plate. This was allowed to harden and was incubated for 48 hours at 30 degrees C. At this time, two areas of swarming (Mot^+) cells appearing to originate from the same point on the plate were seen (areas of diffuse brightness). Discrete colonies of Mot^- cells can also be seen.

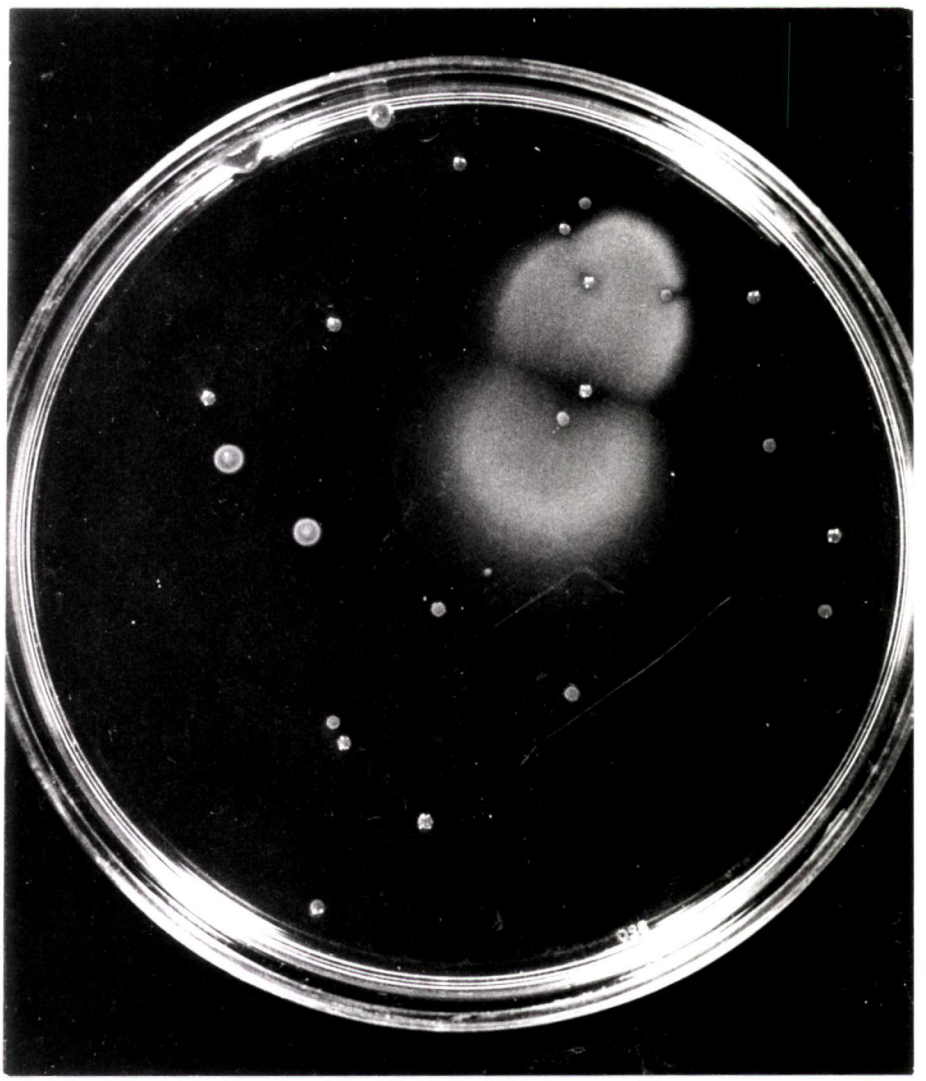


Figure 18. A graph showing that reversion of cells of SA 2940 from Mot^- to Mot^+ is related to cell number.

The protocol is outlined in the text.

The arrow represents the time at which motility first became apparent on a plate containing a 10^{-7} dilution of the organism.

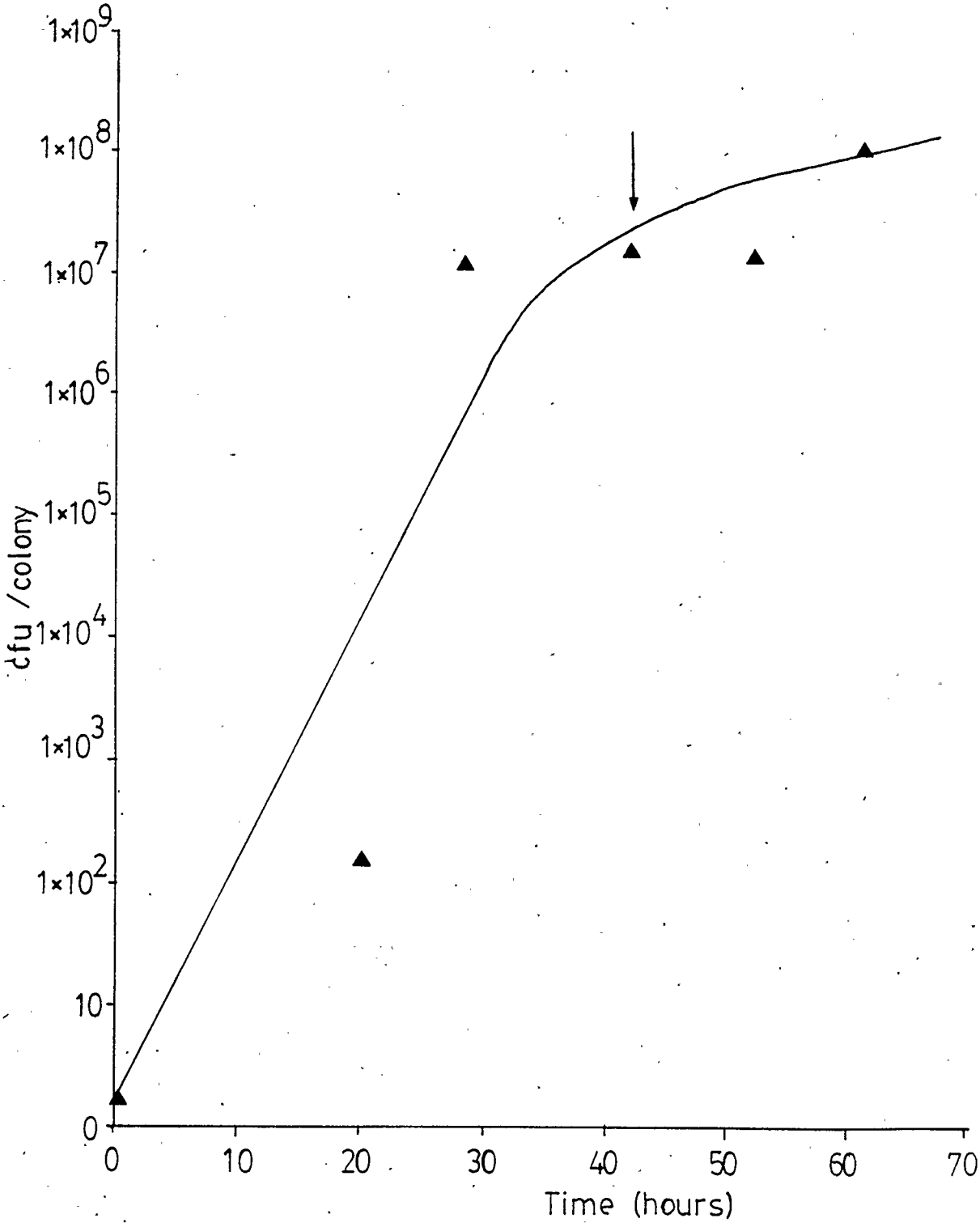
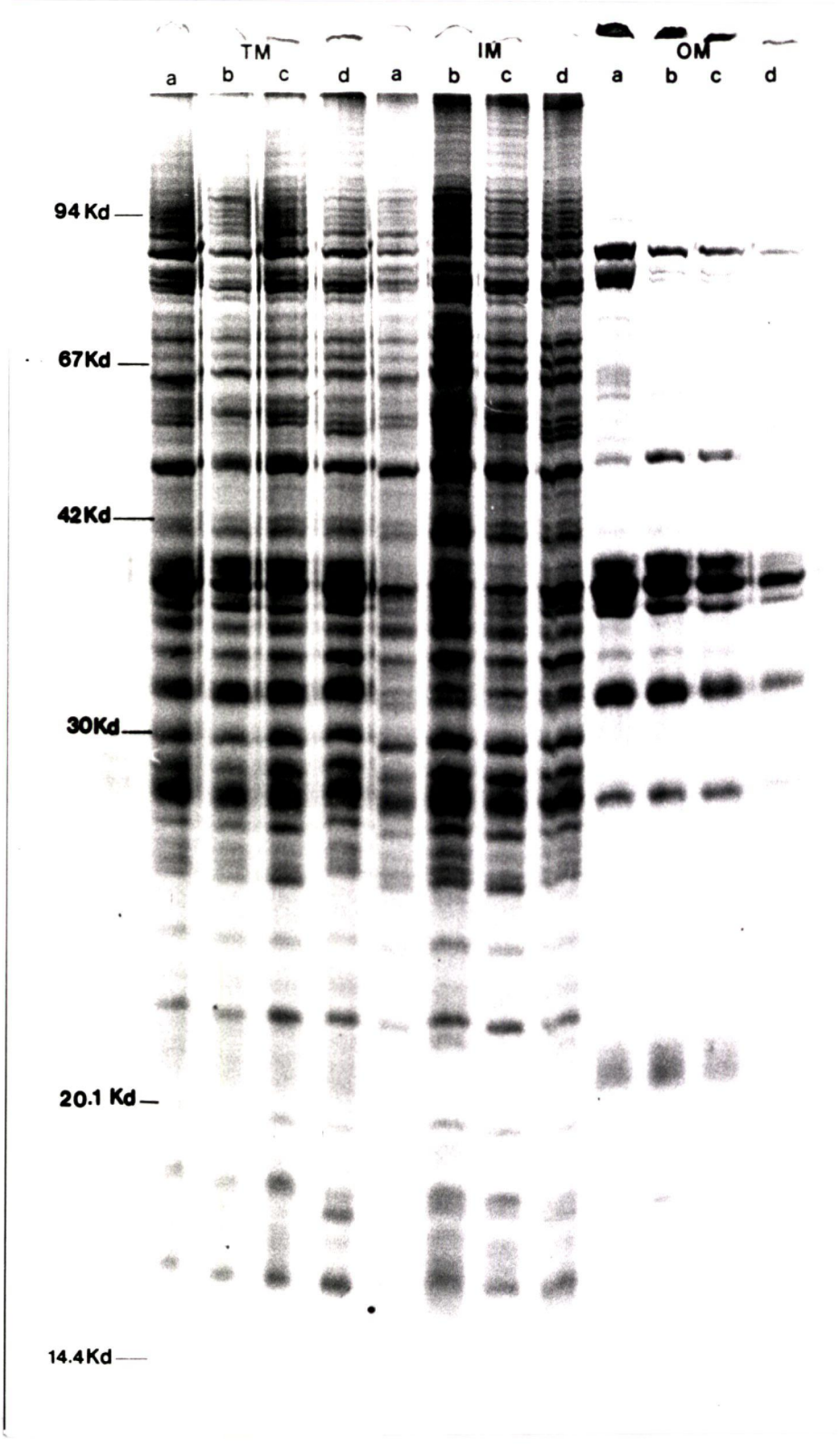

1×10^9
1×10^8
1×10^7
1×10^6
1×10^5
1×10^4
1×10^3
1×10^2
10
0
cfu /colony
0
10
20
30
40
50
60
70
Time (hours)

Figure 19. Autoradiogram of proteins from Mot^+ and Mot^- strains.

Cells were grown and protiens were isolated according to the protocols in Materials and Methods. Approximately 400,000 cpm TM, 300,000 cpm IM, and 200,000 cpm OM were loaded per well. Gels were autoradiographed for 5 days before developing.

Lanes a	SA 2963	smooth Mot^+
Lanes b	SA 2939	smooth Mot^-
Lanes c	SA 2985	smooth Mot^-
Lanes d	SA 2944	smooth Mot^-

Numbers to the left of the gel represent the sizes of protein molecular weight markers which were not labelled with ^{35}S-methionine before running on the gel.


TM
IM
OM
a
b
c
d
a
b
c
d
a
b
c
d
94 Kd
67Kd
42Kd
30Kd
20.1 Kd
14.4Kd