JOURNAL OF BACTERIOLOGY, Apr. 1985, p. 361–366
0021-9193/85/040361-06$02.00/0

Vol. 162, No. 1

Structure of Complex Flagellar Filaments in *Rhizobium meliloti*†

GISELA KRUPSKI, RUDOLF GÖTZ,‡ KURT OBER, EDWIN PLEIER, AND RÜDIGER SCHMITT*

Lehrstuhl für Genetik, Universität Regensburg, D-8400 Regensburg, Federal Republic of Germany

Received 31 May 1984/Accepted 5 December 1984

The complex flagella of *Rhizobium meliloti* 2011 and MVII-1 were analyzed with regard to serology, fine structure, subunits, and amino acid composition. The serological identities of flagellar filaments of the two strains were demonstrated by double immunodiffusion with antiflagellin antiserum. The filaments had a diameter of 16 nm. Their morphology was dominated by the prominent undulations of an external three-start helix running at a 10-nm axial distance and at an angle of 32°. Faint nearly axial striations indicated the presence of a tubular core of a different helical order. The complex filaments consisted of 40,000-dalton flagellin monomers. Typically, the amino acid composition was 3 to 4% higher in nonpolar residues and 5 to 7% lower in aspartic and glutamic acids (and their amides) than that of plain flagellar proteins. There were no immunochemical relationships among *Pseudomonas rhodos*, *Rhizobium lupini*, and *R. meliloti* complex flagella, suggesting that the latter represent a new class.

Rhizobium meliloti 2011 and MVII-1 cells possess 5 to 10 complex flagella (peritrichously inserted) that enable them to move rapidly and respond to tactic stimuli (6). We have previously described and characterized the complex flagella of two other soil bacteria, *Pseudomonas rhodos* (15, 20) and *Rhizobium lupini* H13-3 (5, 19). The filaments are unusual in their fine structure, which is dominated by three conspicuous helical bands, in their fragility (implying rigidity), in their resistance to heat decomposition into monomers, and in their amino acid composition, which exhibits a higher proportion of nonpolar residues than do flagellins of the common plain flagella (14).

R. meliloti flagella are similar in their fine structure to *R. lupini* H13-3 flagella, but they are serologically unrelated. Thus, they represent another example of complex flagella, and we were interested in knowing whether they have the general features characteristic of this flagellar species. Moreover, *R. meliloti* has been extensively studied genetically with respect to chromosomal and plasmid-borne genes responsible for nitrogen fixation and symbiosis with alfalfa (*Medicago sativa* L.) (9, 23). The organism, therefore, is suitable for future analyses of complex flagellum (*fla*), motility (*mot*), and chemotaxis (*che*) genes.

One of the mutants of *R. meliloti* MVII-1 used in the present study overproduces flagella. We purified and compared the flagellar filaments of this strain and of *R. meliloti* 2011. They are serologically cross-reactive and have the fine structure and amino acid composition characteristic of complex flagella.

MATERIALS AND METHODS

Bacterial strains. *R. meliloti* 2011, a streptomycin-resistant derivative of the wild-type strain (2, 17), was obtained from A. Pühler. *R. meliloti* MVII-1 was recently isolated from root nodules of alfalfa (*M. sativa* L.) near Fürth, Federal Republic of Germany (8). Derivative 10406, selected from MVII-1 for high motility on swarm plates, synthesizes roughly twice as many flagella as the parent. Thirteen additional *R. meliloti* strains isolated from root nodules of alfalfa near Cologne, Federal Republic of Germany, were provided by A. Pühler. *R. lupini* H13-3 is an isolate from root nodules of lupine (*Lupinus luteus*) (5).

Media. The synthetic RB medium described previously (6) was used throughout with 0.1% mannitol as the sole carbon source. Swarm plates contained 0.3% agar (Difco Laboratories, Detroit, Mich.), and growth plates contained 1.5% agar (Difco).

Isolation of motile mutants. *R. meliloti* cells grown to the stationary phase were washed, suspended (at 10^9 cells per ml) in RB medium lacking a carbon source and containing 200 μg of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine per ml, and incubated at 30°C for 120 min. After two washes with RB medium, 10-μl samples of suspended cells were placed into the center of swarm plates (RB salts plus 0.1 mM L-proline solidified with 0.3% Bacto Agar [Difco]) (6) and incubated at 30°C for 2 to 3 days. Cells from the periphery of growth were transferred with a toothpick onto fresh swarm plates. After 10 such passages, samples were diluted and spread on RB agar. Single colonies were screened by phase-contrast microscopy (for motility), by the Adler-Pfeffer capillary assay (1, 6), and on swarm plates (for chemotaxis towards L-proline).

Isolation and purification of complex flagella. Motile *R. meliloti* cells were picked from the periphery of growth on swarm plates after 2 days of incubation at 30°C. After 25 h of incubation in 30 ml of minimal medium in a shaker bath, 1-ml samples were spread on agar plates. These were incubated at 30°C for 16 h and subsequently at 25°C for 65 h. Lawns of bacteria were harvested by washing each plate with 2 ml of saline. Flagella were detached from cells by vigorous shaking on a vortex mixer (Heidolph, Kelheim, Federal Republic of Germany) for 15 s, separated from cells by centrifugation at 12,000 × *g* for 10 min, and purified by two series of differential centrifugations as described before (14). The final preparation contained more than 95% flagella, as determined by electrophoresis in 7.5% acrylamide gels in the presence of sodium dodecyl sulfate (21) and by electron microscopy. Typically, 10 plates yielded 0.8 to 1 mg of flagellar protein, as determined by the method of Lowry et al. (12).

Immunodiffusion. Antiserum against purified flagella was prepared from rabbits as previously described (14). For immunodiffusion tests (16), purified flagella (0.5 to 1 mg/ml)

* Corresponding author.

† Dedicated to Wolfram Heumann on the occasion of his 70th birthday.

‡ Present address: John Innes Institute, Norwich, United Kingdom.

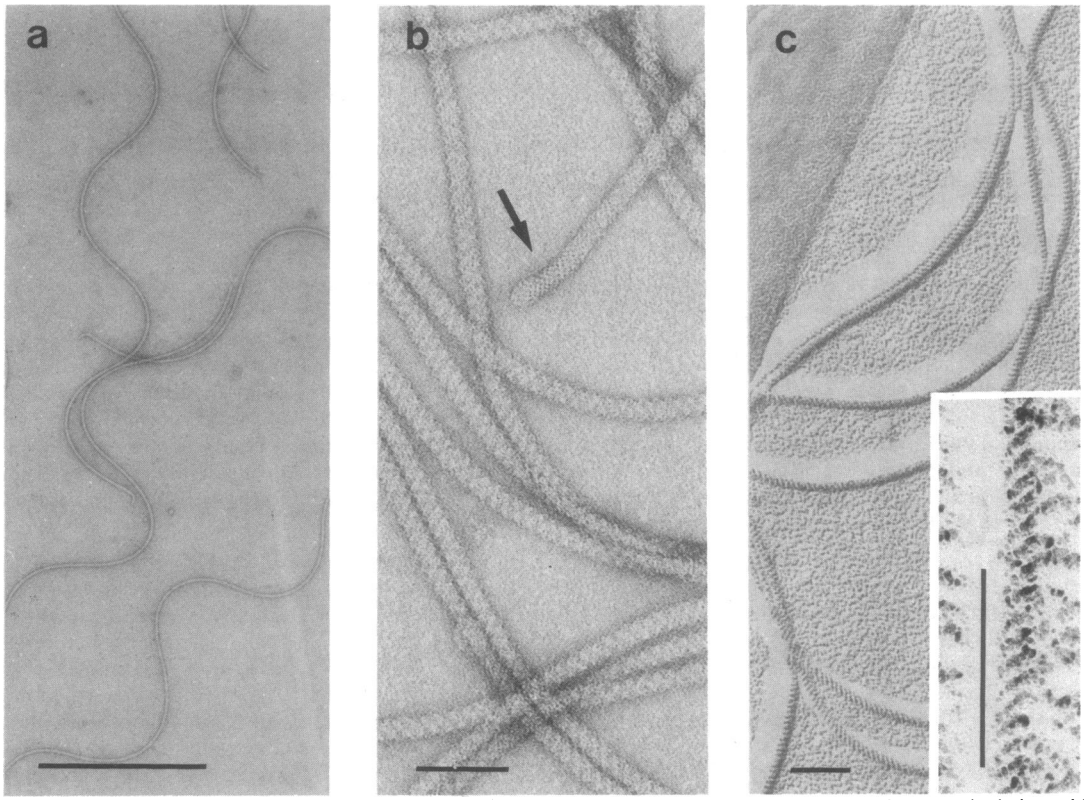

FIG. 1. Electron micrographs of *R. meliloti* flagella. (a) Flagellar filaments of *R. meliloti* MVII-1 showing a typical sinusoidal curvature. Flagella were negatively stained with uranyl acetate. Bar, 1 μm. (b) Isolated complex flagella of *R. meliloti* 10406. The cross-hatched pattern of helical bands in the complex filaments is clearly discernible; the hook structure is indicated by an arrow. Flagella were negatively stained with uranyl acetate. Bar, 100 nm. (c) Metal-shadowed complex flagella of *R. meliloti* 10406. The ropelike filaments exhibit a right-handed helical structure. At a high magnification (inset), these helices appear as alternating elevations and grooves. Bars, 100 nm each.

were treated with 0.2 N HCl (pH 2) at 25°C for 30 min, insoluble material was sedimented (100,000 × *g* for 120 min), and 10-μl samples of the supernatant were applied to wells in an agarose gel (1%).

Determination of amino acid composition. The total amino acid content of purified flagella (40 to 50 μg of protein) was determined with Biotronik A600 and LC5000 automatic amino acid analyzers after hydrolysis in 6 M HCl at 100°C for 48, 60, and 72 h in vacuo. Tryptophan and proline were determined by the methods of Edelhoch (4) and Chinard (3), respectively.

Electron microscopy. For negative staining, 3% uranyl acetate (pH 4.5) was used. Samples were metal shadowed with platinum-iridium at an angle of 10°. Microscope magnifications were calibrated with a diffraction cross-grating replica. Micrographs were taken with a JEM100C electron microscope (Japan Electronic Optics Laboratory, Tokyo, Japan) at 80 kV.

RESULTS

Gross morphology and fine structure of *R. meliloti* flagella. Electron micrographs of negatively stained *R. meliloti* flagella (pH 4.5) revealed a typical sinusoidal curvature (representing the helical structure of the free filament) with an average period of 1.45 μm and an average amplitude of 0.27 μm (Fig. 1A). At higher magnifications, *R. meliloti* flagella exhibited the cross-hatched pattern of helical bands characteristic of complex flagella (Fig. 1B). As for other flagella, their diameters averaged ca. 16 nm. Hooks were distinguishable by a different striation and curvature, their average length being 67 nm and their average diameter being 17 nm.

The morphology of the complex flagella was dominated by the prominent undulations of an external three-start helix. Less obvious were nearly axial lines thought to originate from a tubular core of a different helical order. The dimensions of the three-start helix were determined by evaluating high-resolution micrographs. Helices ran around the filament at a pitch angle of 32° and at an average axial distance of 10 nm. These values (Table 1) were determined with straight portions of 30 filaments; they were in good agreement with data obtained for *R. lupini* H13-3 flagella by optical diffraction (19). The handedness of the three-start helix could not be determined from negatively stained samples, as these showed a superposition pattern of features from the upper and lower sides of each flagellum. To obtain a one-sided image, we used metal shadowing at an angle of 10° (Fig. 1C). The ropelike structures seen in these preparations clearly demonstrated the right-handedness of the helical bands. At a high magnification (Fig. 1C, inset), it became obvious that the surfaces of the complex filaments consisted of alternating elevations (helical bands) and grooves (gaps between helices). This peculiar structure may improve the hydrodynamic properties of the flagellum when it propels the bacterial cell (6).

Serological relationships. *R. meliloti* complex flagella were isolated and purified by differential centrifugation to homogeneity, as determined by gel electrophoresis (21) and by electron microscopy. Rabbit antiserum produced against purified filaments of strain MVII-1 was used for probing

TABLE 1. Structural parameters of flagellar filaments in three *R. meliloti* strains[a]

Strain	Gross morphology of flagella			Fine structure of helical bands			
	Diam (nm)	Length of helical period at pH 4.5 (μm)	Amplitude of helical period at pH 4.5 (μm)	Axial spacing (nm)	Pitch angle (°)	No. of helices[b]	Handedness
2011	17.1 ± 1.2	1.37 ± 0.20	0.31 ± 0.03	10.0 ± 0.2	32 ± 2	3.3	Right
MVII-1	15.5 ± 1.2	1.45 ± 0.15	0.27 ± 0.03	10.0 ± 0.4	31 ± 3	2.9	Right
10406	15.6 ± 1.4	1.56 ± 0.16	0.29 ± 0.03	10.4 ± 0.4	33 ± 2	2.9	Right

[a] Standard deviations were calculated from 30 independent measurements.
[b] Calculated by the method of Labaw and Moseley (10).

relationships among flagella of various *R. meliloti* strains and of *R. lupini* H13-3. Flagella of *R. meliloti* 2011 and 10406 (flagellum-overproducing mutant of MVII-1) produced contiguous precipitation bands in the Ouchterlony double diffusion test (16), whereas *R. lupini* flagella did not react (Fig. 2). Accordingly, the H-antigen determinants of *R. meliloti* strains were identical, but there was no serological relationship with the complex filaments of *R. lupini* H13-3. This was corroborated by the host range of the flagellotropic phage 7-7-1 (11), which infects *R. lupini* H13-3 but not *R. meliloti*. In addition, we serotyped 13 wild-type strains of *R. meliloti* isolated from root nodules of alfalfa by using the immobilization of motile cells by MVII-1 antiserum as an assay (agglutination of flagella). Of these isolates, 12 were immobilized, indicating a cross-reaction; 1 strain had no flagella. All cross-reacting strains were shown by electron microscopy to possess complex flagella. It may be concluded that the possession of a single serotype of complex flagella is a characteristic feature of the species *R. meliloti*.

Size and amino acid composition of *R. meliloti* flagellin. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (21) of purified complex filaments from the three *R. meliloti* strains studied here yielded single bands of identical mobility (Fig. 3). As for *P. rhodos* (20) and *R. lupini* (14, 20), the complex filaments of *R. meliloti* flagella consisted of one type of flagellin monomer. Its relative molecular weight (M_r) was estimated to be 40,000 ± 2,000 with pepsin (M_r, 37,000), ovalbumin (M_r, 43,000), and bovine serum albumin (M_r, 68,000) as reference markers.

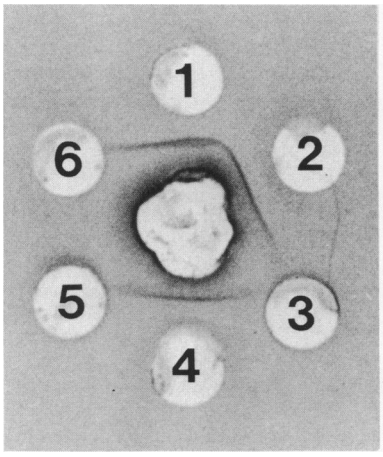

FIG. 2. Ouchterlony double immunodiffusion with rabbit antiserum (central well) prepared against purified flagella of *R. meliloti* MVII-1. The peripheral wells contain the flagellins of *R. meliloti* MVII-1 (well 1), *R. meliloti* 2011 (well 2), *R. lupini* H13-3 (well 3), *R. meliloti* 10406 (well 4), and *R. lupini* H13-3 (well 5); well b contains buffer. Precipitation lines indicate immunochemical cross-reactions.

The amino acid compositions of the flagellin monomers from *R. meliloti* 2011 and 10406, averaged from 5 and 22 independent analyses, respectively, are shown in Table 2. Tryptophan (4) and proline (3) were determined independently; cysteine was not evaluated, as this amino acid is absent from all known flagellar proteins. The compositions of the flagella from the two *R. meliloti* strains were identical within experimental error. Based on the apparent monomer molecular weight of 40,000, both flagellins contained 388 amino acid residues.

DISCUSSION

The flagellar filaments of *R. meliloti* 2011, MVII-1, and derivative 10406 analyzed here had the typical features of complex flagella. Their fine structure, dominated by the prominent undulations of a three-start helix, was strikingly similar to that of the complex flagella of *R. lupini* H13-3 (19), although there was no serological relationship. On the other hand, the filaments of 14 independent isolates of *R. meliloti* were cross-reactive, suggesting that this complex flagellar type may be a common taxonomic feature of *R. meliloti*. A

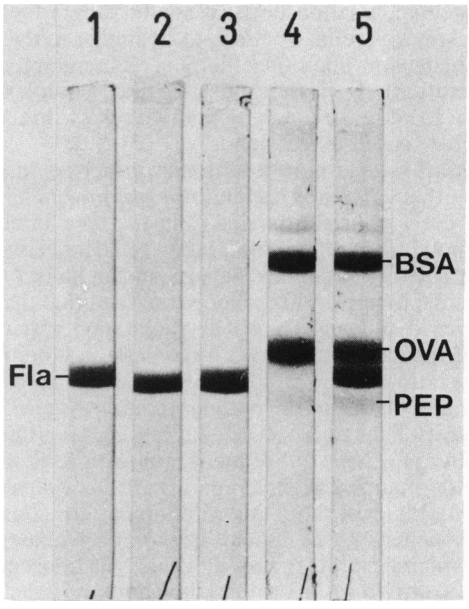

FIG. 3. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of purified dissociated flagella (Fla) of *R. meliloti* 2011 (lane 1), *R. meliloti* MVII-1 (lane 2), and *R. meliloti* 10406 (lane 3); lane 4 contains the standard proteins bovine serum albumin (BSA), ovalbumin (OVA), and pepsin S (PEP), and lane 5 contains flagellin of *R. meliloti* 2011 plus the standard proteins.

TABLE 2. Amino acid compositions of flagellin monomers from *R. meliloti* 10406 and 2011

Amino acid	Mol% of amino acid in strain:	
	10406[a]	2011[b]
Aspartic acid and asparagine	11.89 ± 1.30	12.65 ± 0.97
Threonine	7.48 ± 0.92	7.21 ± 1.21
Serine	10.05 ± 0.97	10.96 ± 1.54
Glutamic acid and glutamine	10.77 ± 0.85	10.10 ± 0.75
Glycine	8.47 ± 0.68	8.45 ± 0.63
Alanine	15.20 ± 1.54	14.76 ± 2.07
Valine	8.80 ± 1.29	7.76 ± 1.36
Methionine	1.74 ± 0.17	1.31
Isoleucine	4.84 ± 0.47	5.07 ± 0.26
Leucine	8.70 ± 0.87	9.38 ± 1.14
Tyrosine	1.79 ± 0.22	1.62 ± 0.07
Phenylalanine	1.85 ± 0.22	1.59 ± 0.15
Histidine	0.53 ± 0.15	0.42 ± 0.10
Lysine	3.74 ± 0.53	4.34 ± 1.28
Arginine	2.96 ± 0.49	3.22 ± 0.84
Proline	0.75 ± 0.40	0.76[c]
Tryptophan	0.44[d]	0.40[d]

[a] Calculated from 22 independent analyses (except for tryptophan).
[b] Calculated from five independent analyses (except for methionine, proline, and tryptophan).
[c] Determined by the method of Chinard (3).
[d] Determined by the method of Edelhoch (4).

scale model of the *R. meliloti* complex filament with structural parameters is shown in Fig. 4. Elevations are distinguished from grooves by shading (boundary lines are somewhat arbitrary). The complete run of one helical band on the cylindrical surface indicated by dotted lines illustrates the actual number of three helices in the filament. This number, calculated by the method of Labaw and Moseley (10) from the diameter, the periodicity of the helices, and the pitch angle of the helices, is in accord with the structures of other complex flagella (19, 20). An inner core structure, suggested by nearly axial striations, was recently shown by three-dimensional image reconstructions to be similar to the arrangement of helices in plain flagella (S. Trachtenberg, personal communication). It is thus possible that complex flagella combine a structural element of plain flagella with the special feature of external helical bands.

R. meliloti flagella have additional properties in common with the other complex species: the prominent three-start helices are always right-handed, and the flagella are highly fragile (implying rigidity) and resistant to heat decomposition (data not shown). Complex flagella so far have only been found in soil bacteria, and it may be inferred that they confer a selective advantage in taxis by facilitating efficient locomotion of their cells (6). Rigid propellers may be of advantage in viscous media, although we recently observed heteromorphous filaments of *R. lupini* complex flagella with a high-intensity light microscope, suggesting a certain degree of flexibility (13). In a subsequent publication (R. Götz and R. Schmitt, manuscript in preparation) we will show that complex flagella have a unique rotation pattern consisting of clockwise spins and of stops. It is conceivable that this pattern, which generates random walks, is a consequence of the specific structure of complex filaments.

Molecular weight determinations and amino acid analyses of purified *R. meliloti* 2011 and 10406 filaments yielded identical results within the limits of the methods used (Table 2). In accordance with results of phage typing and serotyping, the amino acid compositions of *R. lupini* (14) and *R. rhodos* filaments (C. Edelbluth, Ph.D. thesis, University of Erlangen, Erlangen, Federal Republic of Germany, 1974) were different from those of *R. meliloti* filaments, as were the molecular weights of their flagellin monomers. Features of the amino acid compositions that distinguish complex and plain filaments can be derived from data shown in Table 3. The complex species contain higher proportions of nonpolar residues (averaging 37.3% alanine, leucine, isoleucine, and valine) than do plain flagella (ca. 34%). This "deficiency" in the latter is partially balanced by the aspartic and glutamic acid content (or their amides) of 27 to 29.6%, as compared with 19 to 22.6% in the complex species. Presumably, the excess aspartic and glutamic acid residues of plain flagella are accounted for by their neutral amides, as the proportions of basic amino acid residues were similar in both plain and complex flagella and as their isoelectric points were equal (ca. pH 5) (14). For the other groups of amino acids residues listed in Table 3, there were no significant differences between plain and complex flagellar proteins. The proportions of polar residues (threonine, serine, and glycine) were high throughout, the amounts of phenylalanine, tyrosine,

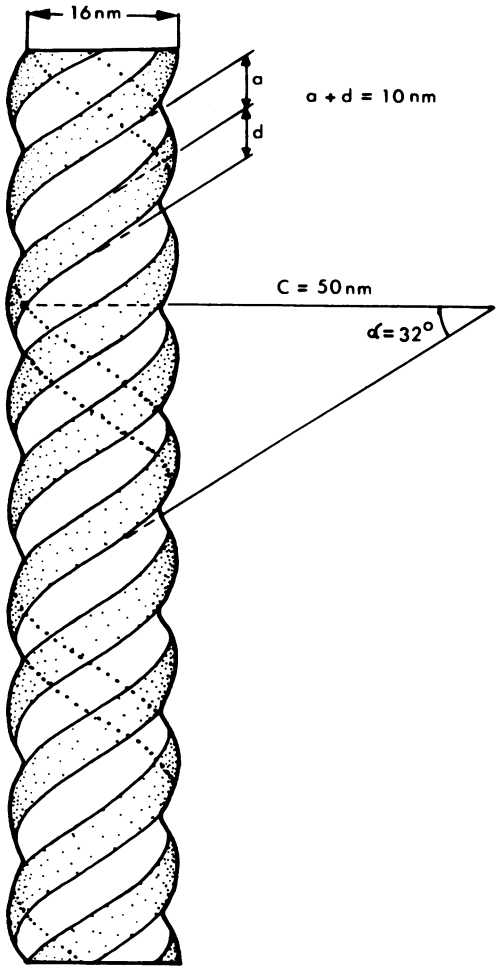

FIG. 4. Scale model of the *R. meliloti* complex flagellar filament as deduced from electron micrographs. The helical surface structure consists of alternating elevations or bands (shaded) and grooves running at an angle (α) of 32°. The three-start helix was derived by a calculation (10) that uses the angular pitch, the axial distance (a + d) of two neighboring helices, and the circumference (C) of the helical cylinder. The run of a single helical band is shown (dotted lines).

TABLE 3. Relative compositions of complex and plain filaments as percentages of nonpolar, polar, acidic, basic, aromatic, and other amino acid residues

Type of residue	Amino acid residues	% of amino acid in:				
		Complex flagella			Plain flagella	
		R. meliloti 10406	*R. lupini* H13-3[a]	*P. rhodos* B9[b]	*Salmonella typhimurium* SJ25[c]	*Bacillus subtilis* 168[d]
Nonpolar	Ala, Val, Ile, and Leu	37.5	37.7	36.7	33.8	34.2
Polar	Thr, Ser, and Gly	26.0	27.5	30.8	25.1	20.1
Acidic	Asp, Glu, Asn, and Gln	22.6	21.7	19.0	27.0	29.6
Basic	Lys and Arg	6.7	8.3	7.9	8.5[e]	9.5
Aromatic	Phe and Tyr	3.6	3.2	3.9	3.9	2.0
Miscellaneous	Met	1.7	2.0	0.4	0.6	2.6
	His	0.5	0	0	0.2	1.3
	Trp	0.4	0	ND[f]	0	0
	Pro	0.8	0	1.2	0.9	0.7
Apparent M_r		40,000	43,000	55,000	56,000	40,000

[a] From reference 14.
[b] From reference 14 and C. Edelbluth, Ph.D. thesis, University of Erlangen, Erlangen, Federal Republic of Germany.
[c] From reference 7.
[d] From reference 22.
[e] Includes *N*-methyllysine.
[f] ND, Not determined.

methionine, and proline were moderate to low, and histidine and tryptophan residues were barely detectable or completely absent from flagellins.

The amino acid composition of *R. meliloti* flagellin published by Schleicher and Bergman (18) differs from our results with regard to single residues and to the proportions of nonpolar and acidic residues thought to be characteristic of complex flagella. It may be possible that these authors used a different strain not possessing complex flagella. On the other hand, we only observed one serotype of complex flagella in 14 motile strains of *R. meliloti*, the identity of *R. meliloti* being confirmed by in vitro nodulation of *M. sativa*. It thus appears that the complex flagella described here are predominant among *R. meliloti* strains.

ACKNOWLEDGMENTS

We thank Alf Pühler for providing bacterial strains and Shlomo Trachtenberg for communicating unpublished results. We are indebted to Helmut König and Gerd Steffens for amino acid analyses.

This investigation was supported by a grant from the Deutsche Forschungsgemeinschaft (SFB4).

LITERATURE CITED

1. **Adler, J.** 1973. A method for measuring chemotaxis and use of the method to determine optimum conditions for chemotaxis by *Escherichia coli*. J. Gen. Microbiol. **74**:77–91.
2. **Brockwell, J., and F. W. Hely.** 1966. Symbiotic characteristics of *Rhizobium meliloti*: an appraisal of the systematic treatment of nodulation and nitrogen fixation interactions between hosts and rhizobia of diverse origins. Aust. J. Agric. Res. **17**:885–899.
3. **Chinard, F. P.** 1952. Photometric estimation of proline and ornithine. J. Biol. Chem. **199**:91–95.
4. **Edelhoch, H.** 1967. Spectroscopic determination of tryptophan and tyrosine in proteins. Biochemistry **6**:1948–1954.
5. **Gabor, M.** 1965. Transformation of streptomycin markers in rough strains of *Rhizobium lupini*. II. The relation between the determinant of streptomycin dependence and those of streptomycin. Genetics **52**:903–913.
6. **Götz, R., N. Limmer, K. Ober, and R. Schmitt.** 1982. Motility and chemotaxis in two strains of *Rhizobium* with complex flagella. J. Gen. Microbiol. **128**:789–798.
7. **Kagawa, H., K. Owaribe, S. Asakura, and N. Takahashi.** 1976. Flagellar hook protein from *Salmonella typhimurium* SJ25. J. Bacteriol. **125**:68–73.
8. **Kamberger, W.** 1979. An Ouchterlony double diffusion study on the interaction between legume lectins and rhizobial cell surface antigens. Arch. Microbiol. **121**:83–90.
9. **Kondorosi, A., G. B. Kiss, T. Forrai, E. Vincze, and Z. Banfalvi.** 1977. Circular linkage map of *Rhizobium meliloti* chromosome. Nature (London) **268**:525–527.
10. **Labaw, L. W., and V. M. Moseley.** 1954. Periodic structure in the flagella and cell walls of a bacterium. Biochim. Biophys. Acta **15**:325–331.
11. **Lotz, W., G. Acker, and R. Schmitt.** 1977. Bacteriophage 7-7-1 adsorbs to the complex flagella of *Rhizobium lupini* H13-3. J. Gen. Virol. **34**:9–17.
12. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
13. **Macnab, R. M., and S.-I. Aizawa.** 1984. Bacterial motility and the bacterial flagellar motor. Annu. Rev. Biophys. Bioeng. **13**:51–83.
14. **Maruyama, M., G. Lodderstaedt, and R. Schmitt.** 1978. Purification and biochemical properties of complex flagella isolated from *Rhizobium lupini* H13-3. Biochim. Biophys. Acta **535**:110–124.
15. **Marx, R., and W. Heumann.** 1962. Über Geisselfeinstrukturen und Fimbrien bei zwei *Pseudomonas*-Stämmen. Arch. Mikrobiol. **43**:245–254.
16. **Ouchterlony, O.** 1967. Immunodiffusion and immunoelectrophoresis, p. 655–706. *In* D. M. Weir (ed.), Handbook of experimental immunology. Blackwell Scientific Publications, Oxford.
17. **Scherrer, A., and J. Dénarié.** 1971. Symbiotic properties of some auxotrophic mutants of *Rhizobium meliloti* and of their prototrophic revertants. Plant Soil, special vol., p. 39–45.

18. **Schleicher, L. R., and K. Bergman.** 1981. The amino acid composition of *Rhizobium meliloti* flagellin: occurrence of a single tryptophan residue. Biochem. Biophys. Res. Commun. **100**:1634–1641.
19. **Schmitt, R., I. Bamberger, G. Acker, and F. Mayer.** 1974. Feinstrukturanalyse der komplexen Geisseln von *Rhizobium lupini* H13-3. Arch. Microbiol. **100**:145–162.
20. **Schmitt, R., I. Raska, and F. Mayer.** 1974. Plain and complex flagella of *Pseudomonas rhodos*: analysis of fine structure and composition. J. Bacteriol. **117**:844–857.
21. **Shapiro, A. O., E. Vinuela, and I. V. Maizel.** 1967. Molecular weight estimation of polypeptide chains by electrophoresis in SDS-polyacrylamide gels. Biochem. Biophys. Res. Commun. **28**:815–820.
22. **Simon, M. I., S. U. Emerson, J. H. Shaper, P. D. Bernard, and A. N. Glazer.** 1977. Classification of *Bacillus subtilis* flagellins. J. Bacteriol. **130**:200–204.
23. **Zimmerman, J. L., W. W. Szeto, and F. M. Ausubel.** 1983. Molecular characterization of Tn*5*-induced symbiotic (Fix^-) mutants of *Rhizobium meliloti*. J. Bacteriol. **156**:1025–1034.