Journal of Industrial Microbiology (1996) 16, 377–382

Flagellar proteins of motile spores of *Actinomycetes*

NI Vesselinova and JC Ensign

Department of Bacteriology, University of Wisconsin, Madison, Wisconsin 53706, USA

Flagella of some of the actinoplanete genera were purified and the molecular sizes of their flagellin subunits compared by SDS-PAGE analysis to flagellins of cells of other bacteria. Several species of *Actinoplanes* have a major flagellar protein of subunit sizes of 42–43 kDa and a lesser amount of a second protein, possibly a minor flagellin subunit, of 60 kDa. The flagellar protein sizes of other actinoplanetes ranged from 32–43 kDa (major) and 48–58 kDa (minor). Antibodies formed against the 42-kDa protein of *A. rectilineatus* showed cross-reactivity in Western blots against flagellar proteins of spores of other *Actinoplanes* species, two species of *Dactylosporangium* and an *Ampullariella* species. Cross-reactivity was also observed with motile cells of two other actinomycetes, *Arthrobacter atrocyaneus* and a *Geodermatophilus* species, and with *Bacillus subtilis*. No cross-reactivity was observed with *Escherichia coli* or *Planomonospora parontospora* flagellar proteins. The amino acid composition and partial N-terminal sequence of the 42-kDa flagellar protein of *A. rectilineatus* was compared to literature data for other bacterial flagellins and found to be most similar to *B. subtilis* 168.

Keywords: motile spores; flagellar proteins of *Actinomycetes*

Introduction

Actinomycetes that produce flagellated and motile spores were first reported in 1955 by Couch [1]. These bacteria, now classified as the genus *Actinoplanes* in the family *Actinoplanaceae* are characterized by being Gram-positive, having DNA with a high G+C content of 68–72%, growing as filamentous branched coenocytic mycelia and reproducing by forming spores. Since this first isolation, many genera of actinomycetes forming spores that are motile and enclosed within a sporangial structure have been described. One important characteristic of the different genera is the number and arrangement of spores in the sporangia. Examples are the genus *Actinoplanes* wherein several hundreds of spores are arranged in linear chains [2], *Dactylosporangium* wherein three spores are in each sporangium [15], and *Planomonospora* with one rod-shaped spore per sporangium [16]. The different genera have a similar life cycle. Spores released from the sporangia either possess flagella or form them rapidly following their release. The spores are actively motile and ultimately germinate to form germ tubes that grow into mycelia. The life cycle is completed with differentiation into sporangia and spores. These sporangiate actinomycetes are the only genera of bacteria known to form intrasporangial motile spores.

Many prosthecate bacteria form motile dispersal cells which undergo differentiation prior to reproduction [3]. The motile spores of three actinomycete genera, *Actinoplanes*, *Dactylosporangium*, and *Planomonospora* can be considered spores since they are less metabolically active and more heat- and desiccation-resistant than the vegetative mycelia (unpublished observations). The rate of endogenous metabolism, measured by rate of oxygen consumption normalized to cell dry weight, of the spores of the three organisms is less than 25% of the mycelia. The spores survived heating at 55°C for 30 min which was lethal to the mycelia. The sporangiate actinomycetes have been recognized in recent years as important producers of antibiotic molecules, some of which are unique molecules [13].

Very little is known of the developmental biology of the sporangiate actinomycetes. Several studies employing electron microscopy have provided an insight into the sporulation process [11,17]. The sporangium wall appears to originate from the outer of a double wall layer of the mycelia. In members of the genus *Actinoplanes*, multiple branching of the mycelium occurs, forming finger-like projections that grow into the sporangium vesicle. These mycelia then divide by formation of annular septa into mono-nucleate units that mature into phase-bright spores. The spores are clearly exospores in the sense of being formed by fragmentation of mycelia. Although the spores are formed inside an outer covering, the sporangial wall, they cannot be considered endospores, which are generally accepted as originating inside the cytoplasm of the mother cell. The spores are released from the sporangia soon after coming in contact with water. The only comprehensive study of spore release and motility was made by Higgins [7] using *Actinoplanes* sp strain 7–10. He reported that spore release involved rupture of the sporangia resulting from swelling of the spores and an intrasporangial material. The spores were non-motile and nonflagellated when released. They acquired flagella and motility 30 min later.

The initial isolations of sporangiate actinomycetes involved baiting techniques using pollen or hair particles [1]. Palleroni [12] described a procedure for isolating motile spores in capillary tubes by taking advantage of their attraction to halide ions. Hayakawa *et al* [6] reported γ-collidine (2,4,6-trimethylpyridine) and xylose to be effective chemoattractants of various genera of actinoplanetes. The basis for chemoattraction to these chemicals is unknown.

This investigation was made for two purposes. One was

Correspondence: JC Ensign, Department of Bacteriology, University of Wisconsin, Madison, Wisconsin 53706, USA
Received 7 February 1996; accepted 1 May 1996

to determine if the flagellar proteins of the actinomycete spores are conserved amongst the different genera and if they are similar to flagellins of motile vegetative bacterial cells. The second purpose was directed to the possibility that characterizing the flagellar proteins might prove useful in devising improved methods for isolation of these important antibiotic-producing bacteria.

Materials and methods

Organisms and growth conditions

The cultures of bacteria studied and media used for their growth are listed in Table 1. *Actinoplanes* isolates 13, 48 and 49 were isolated in this laboratory from a local soil sample. Identification of these isolates was based on microscopic examinations showing spherical sporangia which released motile spores. Solid media were inoculated by spreading Petri dishes containing 25 ml media with 0.2 ml of a spore suspension obtained as described below. To obtain sufficient numbers of spores for flagella isolation, 20 plates were inoculated for each organism. The cultures were grown at 30°C for 5–7 days at which time confluent growth with abundant sporangia had formed. For cultures grown in liquid, 100 ml of broth per 500-ml flask was inoculated with 1.0 ml of a 2-day seed culture and incubated at 30°C while shaken at 120 rpm for 3 days. Exceptions were *Geodermatophilus* which was shaken for 7 days and *Escherichia coli* which was incubated at 37°C for 1 day.

Purification of flagella

Potassium phosphate buffer (10 ml, 0.01 M, pH 7.0) was added to Petri dish cultures and the surface of the plates was gently scraped with a bent glass rod. Motile spores, verified by phase-contrast microscopic examination, were released from sporangia 10–60 min following flooding of the plates. The spores were sedimented by centrifugation at 5000 × *g* for 10 min at room temperature and washed twice with distilled water. These manipulations were done as gently as possible and microscopic examination showed that 90% or more of each spore preparation was actively motile. Cells grown in liquid culture were centrifuged and washed twice with water. Cells and spores were suspended in 10 ml water and deflagellated by gentle sonication using a Branson Model 350 sonifier (Branson Corp, Plainview, NY, USA) with a micro-tip and power setting of 5. A 50% pulse cycle of sonication for 1 min was sufficient to stop motility of at least 90% of the spores and cells. The gentle sonication caused no detectable damage to the spores or cells judging from microscopic examination and normal germination of the sonicated spores and growth of cells when incubated in nutrient media. Intact cells or spores were sedimented by centrifugation at 3000 × *g* for 10 min at 4°C. The supernatant fluid was centrifuged once more as above and then at 60000 × *g* for 2 h at 4°C in a Beckman TL-100 (Palo Alto, CA, USA) ultracentrifuge. Pellet fractions for each organism were pooled and concentrated into one tube and the flagellar filaments were washed by resuspension in distilled water and centrifuged at 60000 × *g* a total of five times. The flagellar protein of *A. rectilineatus* was further purified by bringing the suspension of flagella to 30% saturation of ammonium sulfate followed by stirring it at 4°C for 50 min. The precipitated flagellar protein, which did not dissociate into subunits, was sedimented by centrifugation at 60000 × *g* for 30 min.

Characterization of flagella

The subunit composition of the flagellar proteins was determined using SDS-PAGE [10]. A 12% acrylamide gel was used. Proteins were stained with Coomassie blue. The major flagellar protein of *A. rectilineatus* was characterized further. An SDS-PAGE gel with purified flagella loaded in each well position was electrophoresed and the flagellar protein was electroblotted onto an Immobilon-P membrane (Millipore Corp, Bedford, MA, USA) using a Genie electroblotter at 15 V for 40 min. The membrane was stained with Ponceau S (Sigma Corp). The protein band corresponding to the 42-kDa major flagellar protein was excised and the N-terminal amino acid sequence was determined by automated Edman degradation and PTH-amino acid

Table 1 Bacterial cultures and growth media

Strain	Growth media
Actinoplanes rectilineatus NRRL B-16090	SNY agar
A. brasiliensis ATCC 2584	SC agar
Dactylosporangium aurantiacum NRRL B-8111	SN agar
D. fulvum NRRL B-16292	SN agar
Ampullariella pekinensis NRRL B-16604	SNY agar
Planomonospora parontospora NRRL B-8120	SNY agar
Arthrobacter atrocyaneus ATCC 13752	Nutrient broth
Bacillus subtilis 168 UW	Nutrient broth
Escherichia coli UW	Nutrient broth
Geodermatophilus sp from E Ishiguro	Nutrient broth, glucose
Actinoplanes[a] isolates 13, 48, 19	SNY agar

NRRL, Northern Regional Research Laboratory, Peoria, Illinois; ATCC, American Type Culture Collection, Rockville, Maryland; UW, Department of Bacteriology, University of Wisconsin collection.
[a]Isolated from soil by authors.
SNY agar: 1.0% sucrose (autoclaved separately), 0.1% $NaNO_3$, 0.01% $MgSO_4 \cdot 7H_2O$, 0.05% yeast extract, 2.0 mM KH_2-KH_2PO_4 buffer pH 7.0, 1.5% agar. SN agar is SNY without yeast extract added. SC agar: 1.0% soluble starch, 1.0% salt-free casein hydrolysate (type 1, Sigma Chemical Co, St Louis, MO, USA). Nutrient broth (Difco, Detroit, MI, USA) with 0.5% glucose added after autoclaving separately.

derivative analyses by HPLC. The amino acid composition of the protein was determined by acid hydrolysis followed by ion exchange chromatography and detection with ninhydrin.

Antibody formation and Western blotting

The 42-kDa protein separated by SDS-PAGE and electroblotted onto nitrocellulose was used to raise polyclonal antibodies in rabbits. The protein band containing approximately 100 μg protein was dissolved in 0.5 ml dimethylsulfoxide, mixed with 0.5 ml Freund's adjuvant, and injected subcutaneously at multiple sites into a rabbit. Three booster injections were made at 1-month intervals. Two weeks later, blood was obtained and centrifuged at 5000 $\times$ *g* for 10 min. To remove non-specific antibodies that might have been formed by the rabbit in response to exposure to *E. coli*, 5 ml of intact cells of *E. coli* strain RP3098 (a flagellin-minus mutant obtained from J Adler, University of Wisconsin) grown for 24 h in L-broth medium were incubated with the serum for 3 h at 30°C. Cells with attached antibodies were removed by centrifugation. Cross-reactivity of *A. rectilineatus* flagellar protein antibodies to other flagellin proteins was determined using the Protoblot Western blot AP system (Promega Corp, Madison, WI, USA) following directions of the supplier. For these experiments the antibodies were diluted 1 : 1000.

Electron microscopy

Scanning electron microscopy was performed by critical point drying of specimens on polycarbonate filters in 100% ethanol followed by sputter coating with gold. A JEOL model JSM-13 scanning electron microscope was used. Transmission electron microscopy was carried out using a JEOL model 100KX microscope operated at 100 kV. Samples were placed on formvar-carbon coated grids and were negatively stained by 1% (wt/vol) ammonium molybdate (pH 7.0).

Results and discussion

A. rectilineatus morphology

Electron micrographs of *A. rectilineatus* illustrating sporangia, motile spores and flagella are shown in Figure 1. The sporangia contain linear chains of spores (a). The spores are enclosed in a thin sporangial envelope not visible in the scanning micrograph. The spores possess a polar tuft of flagella (b). An example of a purified flagella preparation is shown in (c); some of the flagella appear to be associated with a hook structure (d). The morphology of the other genera of actinomycetes used in this study is different. Members of the genus *Ampullariella* (which may be a synonym of *Actinoplanes* [5]), form lobate sporangia containing 100–200 motile rod-shaped spores. Members of the genus *Dactylosporangium* produce clusters of finger-like sporangia, each containing three polarly flagellated short rod-shaped spores. Clusters of sporangia, each containing one polarly flagellated rod-shaped spore, are characteristic of the genus *Planomonospora*. The genus *Geodermatophilus* is characterized by a complex life cycle involving rod-shaped cells with rudimentary branching and a coccoid cyst-like stage that liberates small rod-shaped cells that are motile by means of polar flagella [8]. The actinomycete relative *Arthrobacter atrocyaneus* has a life cycle which can involve rod- (sometimes branched) and coccoid-shaped cells. The coccoid cells are flagellated [9].

Size of flagellin proteins

The results of SDS-PAGE analyses are shown in Figure 2 and summarized in Table 2. The flagellar protein samples of each organism were resolved into one or two protein bands. The proteins observed in smaller amounts might be minor flagella components such as filament hooks or cap proteins. They might also be true flagellin proteins. Flagella filaments are usually comprised of one flagellin subunit, but some contain two or more flagellins [18]. The flagella of *A. rectilineatus* contain two proteins with molecular masses of 42 and 60 kDa. The flagellar protein sizes of the *Actinoplanes* strains are similar. *A. brasiliensis* has a single subunit of 43 kDa. Two of the three isolates that appear to be *Actinoplanes* species based on their morphology, strains 48 and 49, have two flagellar proteins of 42 and 60 kDa. Isolate 13 has a single flagellar protein of 42 kDa. The flagella protein sizes of the other organisms are different. Two flagellar subunits of 35 and 55 kDa were observed for *D. aurantiacum*, 35 kDa for *D. fulvum*, 32 and 48 kDa for *A. pekinensis*, and 43 and 58 kDa for *P. parontospora*. The difference in flagellin subunit sizes of the *Actinoplanes* species and *A. pekinensis* is interesting in that the genus *Ampullariella* has been proposed as a synonym of *Actinoplanes* [5].

The flagellar protein of *A. rectilineatus* which contains a major protein subunit of 42 kDa and a minor 60-kDa protein (Figure 3, lane 1) was further purified by ammonium sulfate precipitation. This resulted in loss of the 60-kDa protein (Figure 3, lane 3). The 42- and 60-kDa proteins were consistently observed in analyses of several independent flagella filament preparations. The nature of the 60-kDa protein removed by ammonium sulfate precipitation is not known. This protein may be the hook flagellin protein visible in the purified filaments not precipitated by ammonium sulfate (see Figure 1, c and d). The 42-kDa protein is clearly the flagella filament protein since it is sedimented by high speed centrifugation.

Western blot analyses

Antibodies produced against the 42-kDa flagellar protein of *A. rectilineatus* were used to determine immunological similarity of different flagellar proteins. The antibodies showed cross-reactivity with all the spore flagellar proteins except for *P. parontospora* (Figure 4). None of the larger flagellar proteins (present in minor amounts) observed for *A. rectilineatus*, *A. pekinensis* or *Actinoplanes* isolates 48 and 49 reacted with the antibodies. It is likely that these large proteins associated with the flagella preparations are not flagellins; they may be hook structure proteins. The flagellar proteins of cells of two non-sporulating actinomycetes, *Geodermatophilus* sp and *A. atrocyaneus*, showed cross-reactivity with the antibodies. The motile cells of *A. atrocyaneus* are not spores in the sense that they are not dormant and divide by binary fission. They are phylogenetically quite distinct from *Actinoplanes* [4]. The

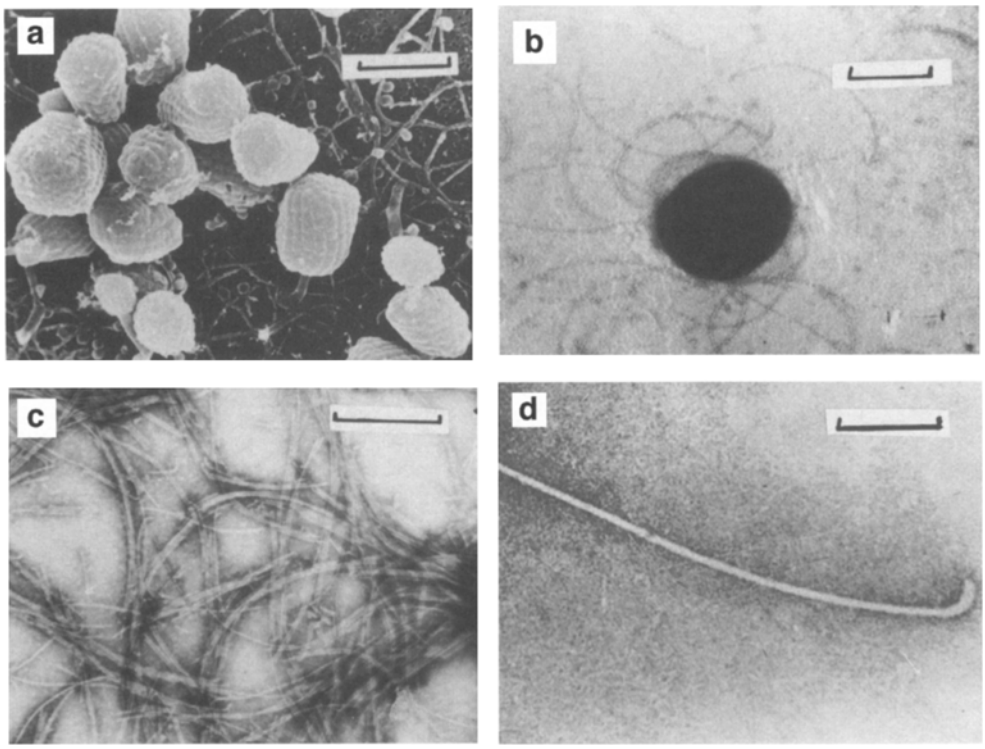

Figure 1 Morphology of *Actinoplanes rectilineatus* spores and flagella. (a) Scanning electron micrograph of colony surface showing mycelia and sporangia. (b) Electron micrograph of negative-stained spores showing polar tuft of flagella. (c) Negative stain of purified flagella. (d) Negative stain showing flagellum filament with hook structure. Bar presents 10 μm (a, b) or 1.0 μm (c, d).

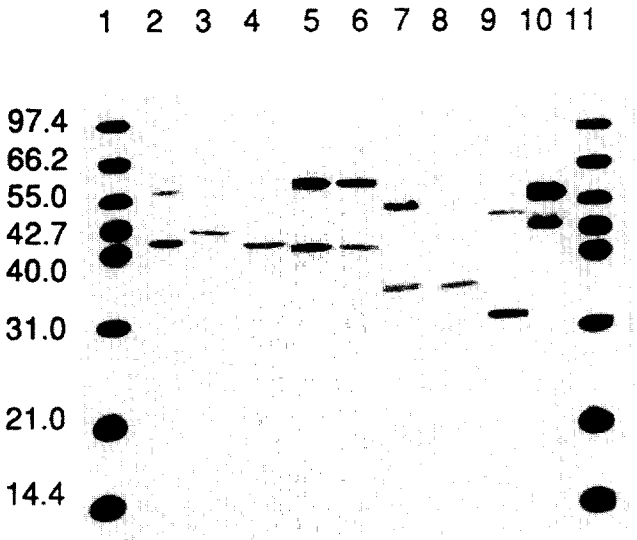

Figure 2 SDS-PAGE analysis of flagellar proteins. Flagella were purified from spores of different genera of Actinoplanaceae. The flagellin proteins were separated in 12% acrylamide gel and stained with Coomasie blue. Lanes 1 and 11, standard proteins (kDa); lane 2, *Actinoplanes rectilineatus*; lane 3, *Actinoplanes brasiliensis*; lanes 4, 5, 6 *Actinoplanes* isolates 13, 48, 49; lane 7, *Dactylosporangium aurantiacum*; lane 8, *Dactylosporangium fulvum*; lane 9, *Ampullariella pekinensis*; lane 10, *Planomonospora parontospora*.

Table 2 Molecular weights of flagellar proteins of spores of different genera of *Actinoplanaceae*

Organism	Flagellar subunit sizes (kDa)
Actinoplanes rectilineatus	42,60
Actinoplanes brasiliensis	43
Dactylosporangium aurantiacum	35,55
Dactylosporangium fulvum	35
Ampullariella pekinensis	32,48
Planomonospora parontospora	43,58
Actinoplanes isolate 13	42
Actinoplanes isolate 48	42,60
Actinoplanes isolate 49	42,60

motile stage cells of *Geodermatophilus* have not been studied and so whether they are vegetative cells or spores is not known. The antibodies react strongly with a 42-kDa flagellar protein and less strongly with a 35-kDa flagellar protein of *B. subtilis*, but did not react with flagellin of *E. coli*. We have no explanation for the low level of antibody reaction with several *B. subtilis* and *E. coli* proteins. It is possible that some degradation of these flagellins occurred during preparation resulting in cross-reactive peptides.

The failure of the *A. rectilineatus* flagellar antibodies to cross-react with *P. parontospora* flagellar protein is interesting. A comprehensive study of DNA homologies and 16S RNA sequences showed that the *Actinomycetales* group into many distinct phyletic clusters [4,14]. One cluster contains *Actinoplanes*, *Ampullariella*, *Dactylosporangium* and several other genera. Goodfellow *et al* [5] proposed, based on phenotypic properties analyses, that these genera should be classed in the family *Micromonosporaceae*. The genus *Planomonospora* belongs to a second dis-

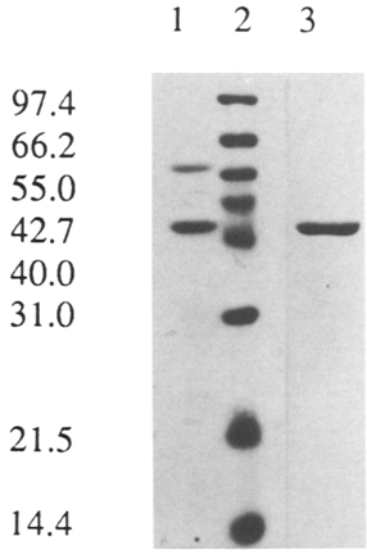

Figure 3 SDS-PAGE analysis of *A. rectilineatus* flagella purified by ammonium sulfate precipitation. Lane 1, flagella prepared by centrifugation and washing. Lane 2, molecular size markers, kDa. Lane 3, ammonium sulfate-purified flagella.

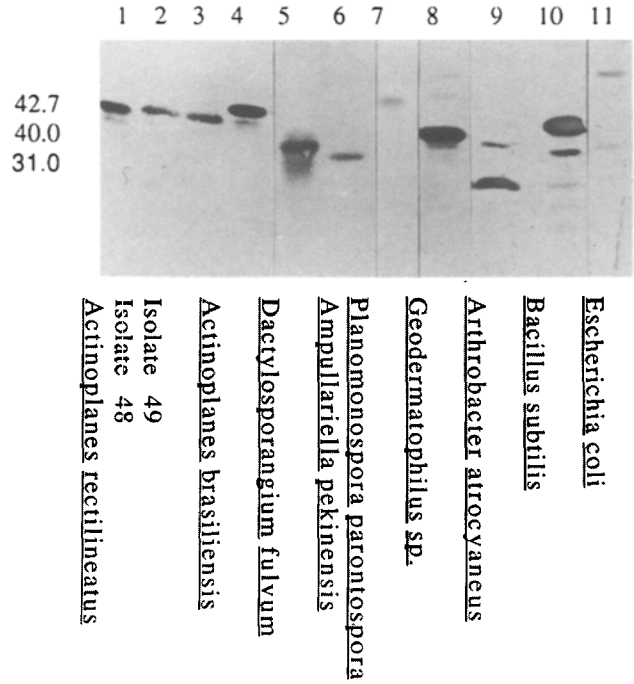

Figure 4 Western blot analysis of cross-reactivity of *Actinoplanes rectilineatus* flagellin antibody with flagellin proteins of different bacteria. The flagellin proteins were separated by SDS-PAGE, transferred to a nitrocellulose membrane, and reacted with antibodies produced against the 42-kDa flagellin protein of *A. rectilineatus*. Molecular sizes (left) in kilodaltons.

tinct cluster in the family *Streptosporangiaceae*. N-terminal amino acid analysis of the flagellin protein of *Planomospora* showed little similarity to the N-terminal amino acids of flagellin of *A. rectilineatus* (Y Takahashi, Kitasato Institute, Tokyo, Japan, unpublished data).

Amino acid sequence of A. rectilineatus flagellar protein

The amino acid composition of the 42-kDa flagellar protein was similar to other bacterial flagellins [18] in being rich in aspartic acid, threonine, serine, glutamic acid, glycine, valine, alanine and leucine and in lacking cysteine and tryptophan. The amino acid composition of the protein (number of amino acids per molecule) is: lysine 12, histidine 1, arginine 18, aspartate/asparagine 53, threonine 37, serine 35, glutamate/glutamine 41, proline 5, glycine 44, alanine 51, valine 28, methionine 9, isoleucine 17, leucine 33, tyrosine 7, and phenylalanine 8.

The partial N-terminal amino acid sequence of *A. rectilineatus* and several other bacterial flagellar proteins is shown in Table 3. In this limited region of the molecule, *A. rectilineatus* flagellin has 10 amino acids in common with flagellins of *B. subtilis* and *Campylobacter jejuni*, seven amino acid positions common to *Caulobacter crescentus*, and only two or four in common with the other bacterial flagellins listed in the Table. This is interesting since antibodies against the *A. rectilineatus* flagellar protein show cross-reactivity with the *B. subtilis* but not *E. coli* flagellins (Figure 4). Flagella of *C. jejuni* were not available for testing. Antibodies to the *A. rectilineatus* flagellar protein blocked motility of the organism's spores and also vegetative cells of *B. subtilis* but did not affect motility of *E. coli* cells (data not shown).

Based on these data, it can be deduced that the 42-kDa flagellar protein of *A. rectilineatus* is not grossly different from flagellins of other bacteria. Amino acid positions 4, 5 and 7 (I, N, N) are highly conserved in bacterial flagellins and so indicate that the flagella protein of *A. rectilineatus* is a true flagellin. It appears that the proteins involved in flagellar motility of bacteria are relatively conserved in evolution, in both motile vegetative cells and the motile spores of sporangiate actinomycetes. The immunological data show that the flagellar proteins of the different sporangiate actinomycete species are similar, with the interesting exception of *P. parontospora* which did not react with antibodies to *A. rectilineatus* (Figure 4). Molecular analyses of actinomycetes revealed that *Planomonospora* and *Actinoplanes* belong to different families (*Streptosporangiaceae* and *Actinoplanaceae* respectively) of the actinomycete phylogenetic line [4,14]. Goodfellow *et al* proposed that the family name *Micromonosporaceae* has precedence over *Actinoplanaceae* [5].

Interesting questions are how flagella synthesis genes are regulated since their expression appears to occur late in sporulation after spores are formed in the sporangia, and if the sporangiate spores, which show chemotactic behavior, have sensory mechanisms similar to other bacteria. These questions are being pursued in our continued work. We have conjugated the *A. rectilineatus* antibodies to magnetic polystyrene beads and demonstrated that the beads will attach to and magnetically remove actinoplanete spores from suspensions. The antibody beads have not yet proven useful in isolating motile spores from natural sources. It is possible that metal ions or other materials in soil and sediment slurries interfere with the antigen-antibody reaction, or that there are too few motile spores present in the samples for attachment to the beads. Attempts to exploit

Table 3 Partial N-terminal amino acid sequences of flagella proteins of *Actinoplanes rectilineatus* and other bacteria[a]

Organism	Amino acid position 1				5					10					15				20
Actinoplanes rectilineatus		**G**	**L**	**R**	**I**	**N**	**Q**	**N**	**I**	**A**	**A**	**Q**	**N**	**A**	**Y**	**R**	**N**	**L**	**S**	**V**	**T**
Bacillus subtilis		–	M	**R**	**I**	**N**	H	**N**	**I**	**A**	**A**	L	**N**	T	L	N	R	**L**	**S**	S	N
Campylobacter jejuni		G	F	**R**	**I**	**N**	T	**N**	V	**A**	**A**	L	**N**	**A**	K	A	**N**	A	D	L	N
Escherichia coli		A	Q	V	**I**	**N**	T	**N**	S	L	T	L	I	T	Q	N	**N**	I	N	K	N
Pseudomonas aeruginosa		A	**L**	T	V	**N**	N	I	A	S	L	N	T	Q	R	N	L	N	N	S	S
Rhizobium meliloti	T	S	I	L	T	**N**	N	S	A	M	**A**	A	L	S	T	L	R	S	I	S	S
Caulobacter crescentus	M	A	**L**	S	V	**N**	T	**N**	Q	P	**A**	L	I	**A**	L	Q	**N**	**L**	N	R	**T**

[a]Sequences for all but *A. rectilineatus* taken from Wilson and Beveridge [18].

the antibody-conjugated beads for actinoplanete isolation are continuing.

Acknowledgements

This work was supported by College of Agriculture and Life Sciences, University of Wisconsin and by a grant from Merck Pharmaceutical Company.

References

1 Couch JN. 1950. *Actinoplanes*, a new genus of the *Actinomycetales*. J Elisha Mitchell Scientific Soc 66: 67–92.
2 Couch JN. 1963. Some new genera and species of the *Actinoplanaceae*. J Elisha Mitchell Scientific Soc 79: 53–70.
3 Dow CS, R Whittenbury and NG Carr. 1983. The shut down or growth precursor cell—an adaptation for survival in a potentially hostile environment. Symp Soc Gen Microbiol 24: 187–247.
4 Embley TM and E Stackebrandt. 1994. The molecular phylogeny and systematics of the actinomycetes. Ann Rev Microbiol 48: 257–289.
5 Goodfellow M, LJ Stanton, KE Simpson and DE Miniken. 1990. Numerical and chemical classification of *Actinoplanes* and some related actinomycetes. J Gen Microbiol 136: 19–36.
6 Hayakawa M, MT Tamura and H Nonomura. 1991. Selective isolation of *Actinoplanes* and *Dactylosporangium* from soil using γ collidine as the chemoattractant. J Ferment Bioeng 72: 426–432.
7 Higgins M. 1967. Release of sporangiospores by a strain of *Actinoplanes*. J Bacteriol 94: 495–498.
8 Ishiguro EE and RS Wolfe. 1970. Control of morphogenesis in *Geodermatophilus*: ultrastructural studies. J Bacteriol 104: 566–580.
9 Kuhn DA and MP Starr. 1960. *Arthrobacter atrocyaneus* n sp and its blue pigment. Arch Microbiol 36: 175–181.
10 Laemmli UK. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (Lond) 227: 680–685.
11 Lechevalier H and PE Holbert. 1965. Electron microscope observation of the sporangial structure of a strain of *Actinoplanes*. J Bacteriol 89: 217–222.
12 Palleroni N. 1980. A chemotactic method for isolation of *Actinoplanaceae*. Arch Microbiol 128: 53–55.
13 Parenti F and C Coronelli. 1979. Members of the genus *Actinoplanes* and their antibiotics. Annu Rev Microbiol 33: 389–411.
14 Stackebrandt EB, E Wunner-Fussl, VJ Fowler and KH Schleifer. 1981. Deoxyribonucleic acid homologies and ribosomal ribonucleic acid similarities among spore forming members of the order *Actinomycetales*. Int J Syst Bacteriol 31: 420–431.
15 Thiemann JE, H Pagani and G Beretta. 1967. A new genus of the *Actinoplanaceae*: *Planomonospora* gen nov. Giornale di Microbiologica 15: 27–38.
16 Thiemann JE, H Pagani and G Beretta. 1967. A new genus of *Actinoplanaceae*: *Dactylosporangium* gen nov. Arch Mikrobiol 58: 42–52.
17 Williams ST and GP Sharples. 1976. *Streptosporangium corrugatum* sp nov, an actinomycete with some unusual morphological features. Int J Syst Bacteriol 26: 45–52.
18 Wilson DR and TJ Beveridge. 1993. Bacterial flagella filaments and their component flagellins. Can J Microbiol 39: 451–472.