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Spirality of Cell Bodies in Genus Spirillum and Genus Rhodospirillum under Scanning Electron Microscopy

Zensaku Yoshii, Hisanori Konishi, Hisaji Akitomi and Akira Mizuno

Department of Microbiology, Yamaguchi University School of Medicine, Ube, Yamaguchi 755, Japan

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Abstract The spirals of cell bodies of 10 species of Genus *Spirillum* and one strain of *Rhodospirillum rubrum* were examined by scanning electron microscopy. The critical point drying technique (CPD) provided more complete information on the cell body than the air drying technique (AD) with respect to regularity of helix, completeness of turn, and spiral-direction. The bacteria in this experiment were classified into two major groups: with and without regular spiral of the cell body. Spiral bacteria were further divided into three groups according to the type of spirality; right-handed turn (*S. hiroshimense, S. multigloburiferum, S. petaginum*, Marine spirillum Watanabe strain); left-handed turn (*S. itersonii, S. peregrinum, R. rubrum*); and having both right- and left-handed spirality (*S. serpens*). Thus findings under scanning electron microscopy will be valuable for the taxonomy of hellically shaped bacteria.

Key Words: Bacteria; spirilla, spirality, Electron microscopy; scanning

Introduction

All the morphological features of helical bacteria, such as Genus *Spirillum* and Genus *Rhodospirillum*, have been studied by light microscopy (LM) and transmission electron microscopy (TEM)[1-11], but not by scanning electron microscopy (SEM). The spiral-direction of the cell body has been ambiguous in previous reports because they were concerned with the two-dimensional features. SEM provided an opportunity to examine the spiral-directions in three dimensional models. In this report we describe SEM observations on ten helical bacteria.

Materials and Methods

Strains. Bacterial strains used in this experiment were as follows: *Aquaspirillum; Spirillum serpens* ATCC 12638, *S. itersonii* ATCC 12639, *S. metamorphum* ATCC 15280, *S. lunatum* IFO 3985, *S. psychrophilum* IFO 13611, *S. peregrinum* IFO 16317, *Oceanospirillum; S. hiroshimense* IFO 13616, *S. multigloburiferum* IFO 13614, *S. petaginum* IFO 13512, marine spirillum Watanabe strain, *Rhodospirillum; R. rubrum* IFO 3986.

Cultures. These strains were cultivated in nutrient

broth at 25°C or 30°C for 24 h.

Specimen preparation for SEM. After harvesting, the cells were washed three times, resuspended in saline solution, and then treated by the double fixation methods[12] with 2% glutaraldehyde and 1% osmic acid. The fixed materials were washed twice in distilled water by centrifugation at 3,000 xg, dehydrated through an acetone series, and dried in air (air drying, AD) or with critical point drying technique (CPD) using the small filter paper envelope method[13]. The dried cells on the glass slides or filter paper were coated with carbon and Au-Pd alloy in an evaporator.

Scanning electron microscopy. The prepared specimens were set in the SEM (JSM-S1 & JSM-F7, JEOLCO, Tokyo) and observed at an accelerating voltages of 10kV or 7kV. The SEM in our laboratory provided an orthogonal image, not a mirror image as in the previous paper[14]. More than 300 cells from each strain were usually observed using stereographic technique. The final decision on spiral-direction was made by criteria previously described[14]. The definition of spiral-direction was based on the proposal of Doty[15] and of Kihara[16].

Observations

All air-dried (AD) bacterial specimens showed a body with imperfect spirals or a simple wavy form. Critical point drying specimens, however, provided good images with perfect spiral. Fig. 1 and Fig. 2 show typical photographs of a marine spirillum with the AD and CPD technique, respectively. Depending on specimens, sizes and shapes varied. The longer cells usually showed a complete turn in the cell body (Fig. 2, arrow a), and the shorter cells an incomplete turn (Fig. 2, arrow b). Therefore, the longer bacteria with well-formed spirals were sought in CPD-specimens and photographed. These observations are summarized in Table 1. The spiral-direction of a particular strain was constant. Three species were always left-handed, while three were right-handed. The direction of the rest three species were unclear. However, one strain, *S. serpens*, showed two different spiral-directions, right- and left-handed with a complete turn.

Discussion

Specimen preparation.

The two methods of drying, AD and CPD, were compared and CPD was far superior to AD (Fig. 1 & 2) for preservation of the

Table 1 Spiral-direction of Cell Bodies of Examined Bacteria

Group		Strains	Spiral-direction	Turn	Figure number
A	1	*S. serpens*	Lh	Usually complete	3
			Rh	Not as good as above	4
	2	*S. itersonii*	Lh	Usually complete	5
	3	*S. metamorphum*	?	Short body, incomplete	6
	4	*S. lunatum*	?	Short body, incomplete	7
	5	*S. psychrophilum*	?	Short body, incomplete	8
	6	*S. peregrinum*	Lh	Usually complete	9
O	7	*S. hiroshimense*	Rh	Usually complete	10
	8	*S. multigloburiferum*	Rh	Usually complete	11
	9	*S. petaginum*	Rh	Usually complete	12
	10	Marine spirillum Watanabe strain	Rh	Usually complete	1 & 2
P	11	*Rhodospirillum rubrum*	Lh	Usually complete	13

A=*Aquaspirillum*, O=*Oceanospirillum*, P=*Photobacterium*;
Lh=left-handedness, Rh=right-handedness, ?=Uncertain

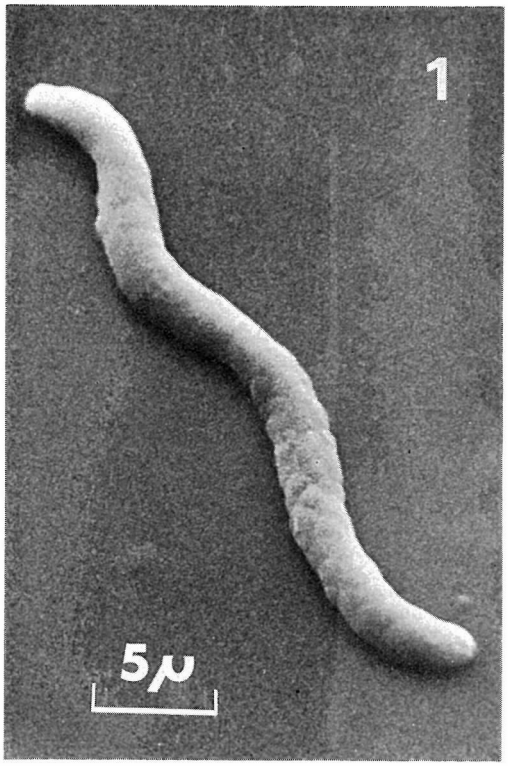

Fig. 1

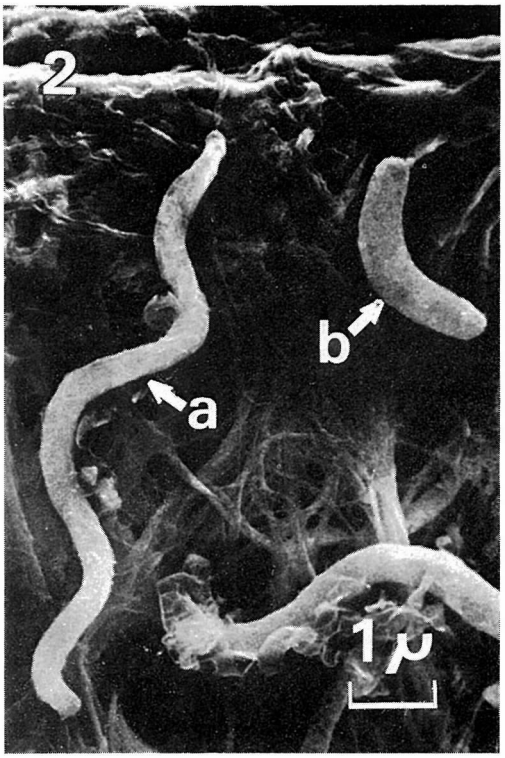

Fig .2

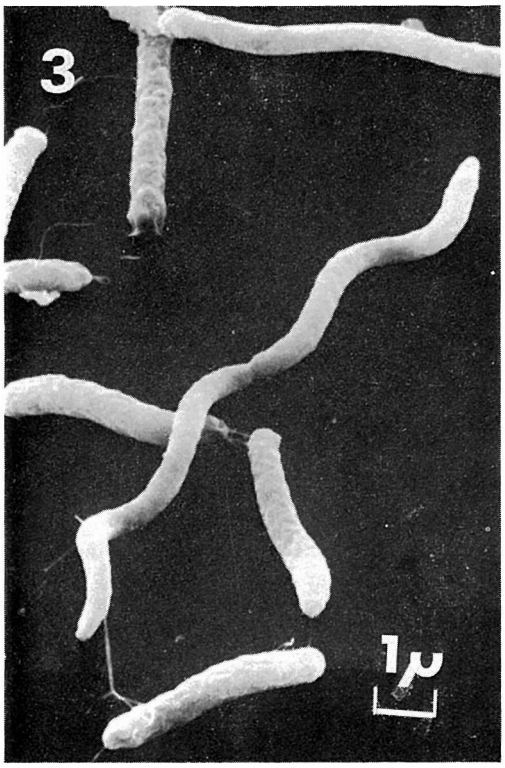

Fig. 3

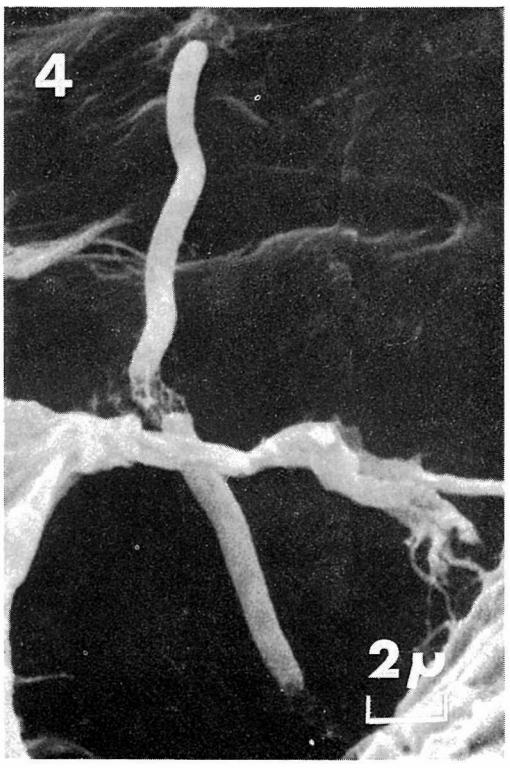

Fig. 4

spiral form of cell bodies (Figs. 2-13). It was clear that the rigidity of the spirilla cell bodies could not be well preserved when cells were air-dried. The findings were consistent with our previous studies on spirochete[17,18]. Therefore, CPD was used in the present study.

Determination of spiral-direction.

In this study, the spiral-directions were consistent with those in the previous paper [14]. There are a number of LM studies for spirilla[6,10,11], but they did not reported on spiral-directions and on methodological problems.

Terasaki[6] and Krieg[11] described the clockwise pattern of *S. serpens*, but our data using SEM showed that the cell bodies of this spirillum had both right- and left-handed spiral-direction. The reasons on these differences are not certain, however, the superior resolving power of SEM probably allow more confident observations.

The consistency of the spiral-direction, either right- or left-handed, was apparent in a particular bacterial strain, except for *S. serpens* strain. In the case of spirochetes, Genus *Leptospira* always showed right-handedness[17] and *Treponema pallidum* Nichols strain always showed left-handedness[18]. But the cell bodies of *T. phagedenis* biotype Reiter showed both right- and left-handed spirals[18]. Yoshii and coworkers[18] reported that these observations would be helpful in bacterial taxonomy, as well as in locomotive physiology of these bacteria. In the present study, we also would like to have the same consideration on the significance of the spiral-direction of helical bacteria as the previous papers[17,18].

The spiral-directions of three strains of spirilla species, *S. metamorphum*, *S. lunatum* and *S. psychrophilum* could not be decided because of incomplete turns in their small cell bodies. This point will be studied in the future.

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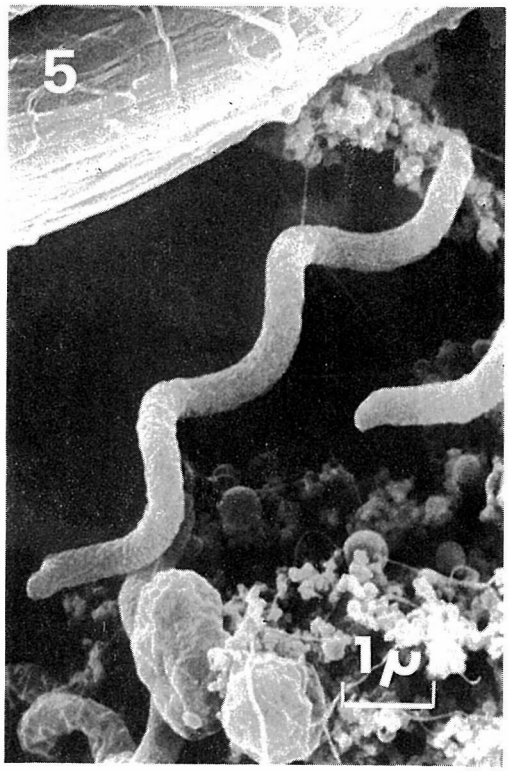
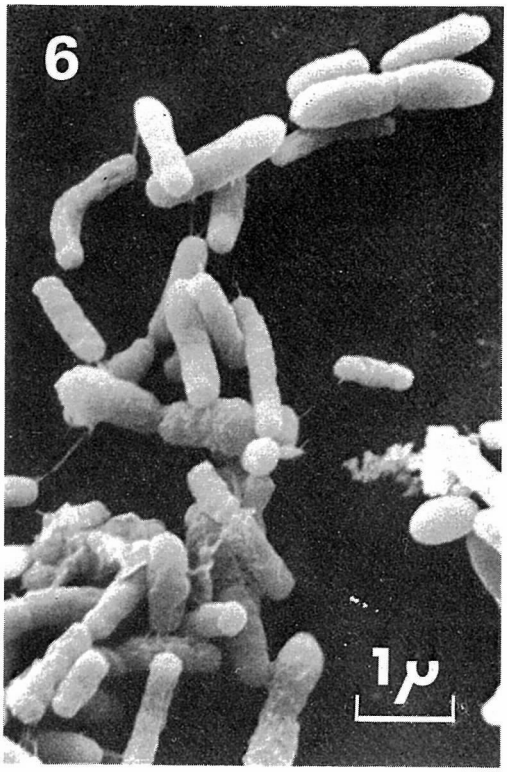


Fig. 5



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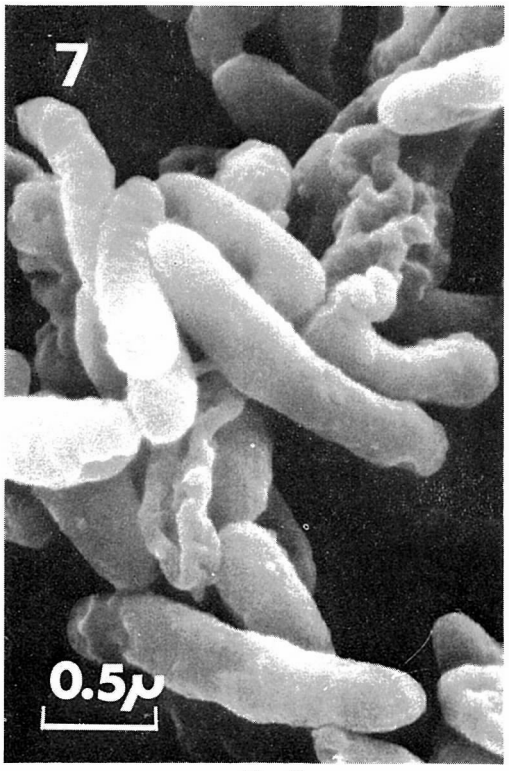

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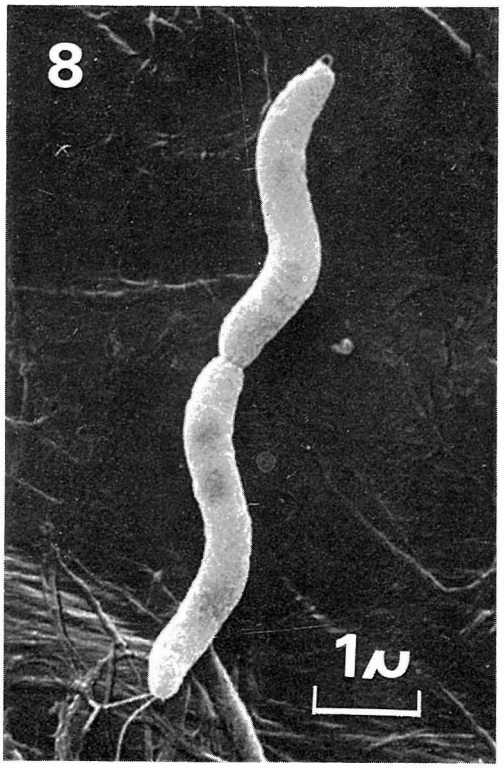

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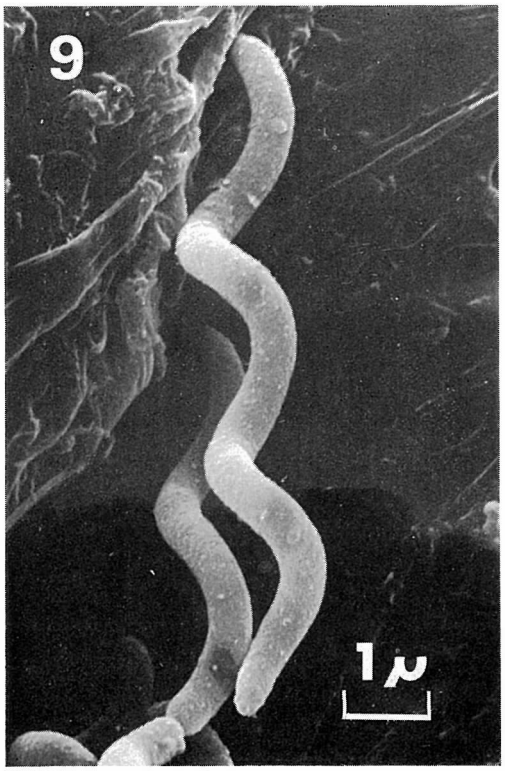

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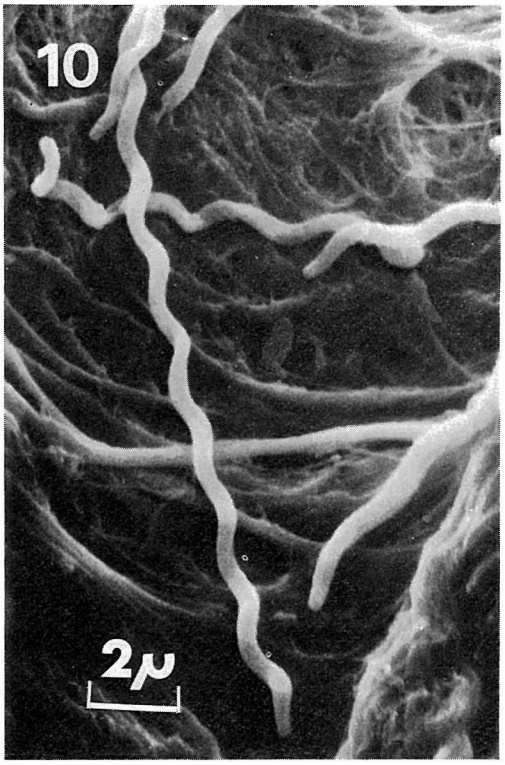

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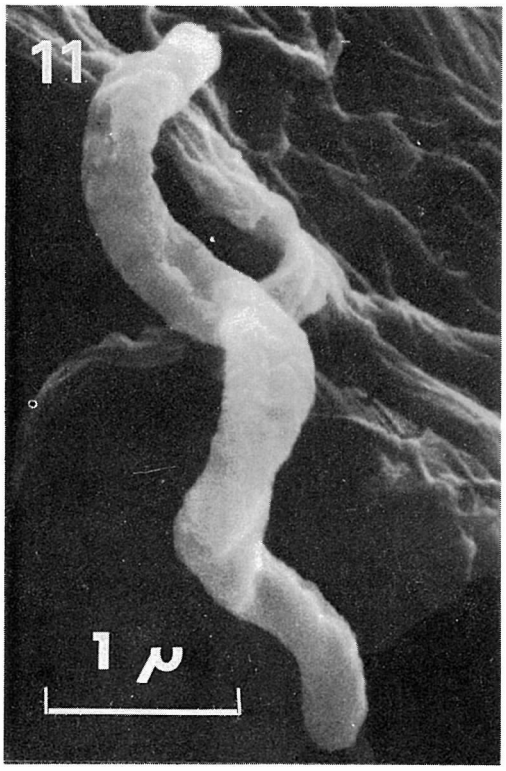

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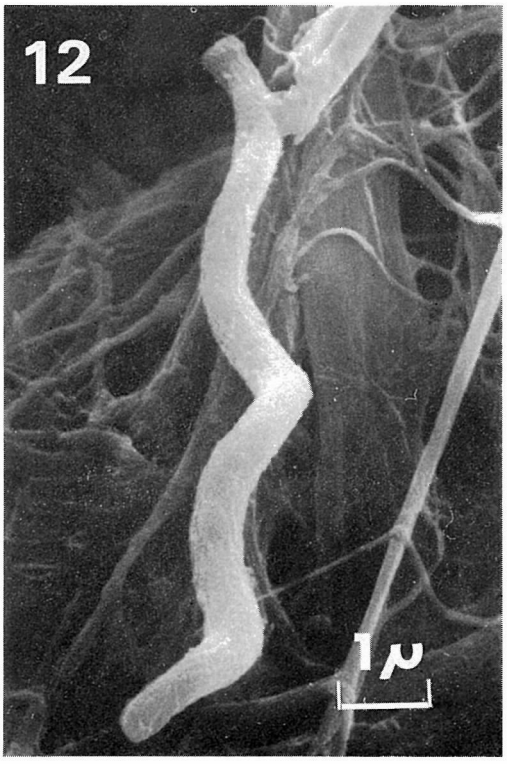

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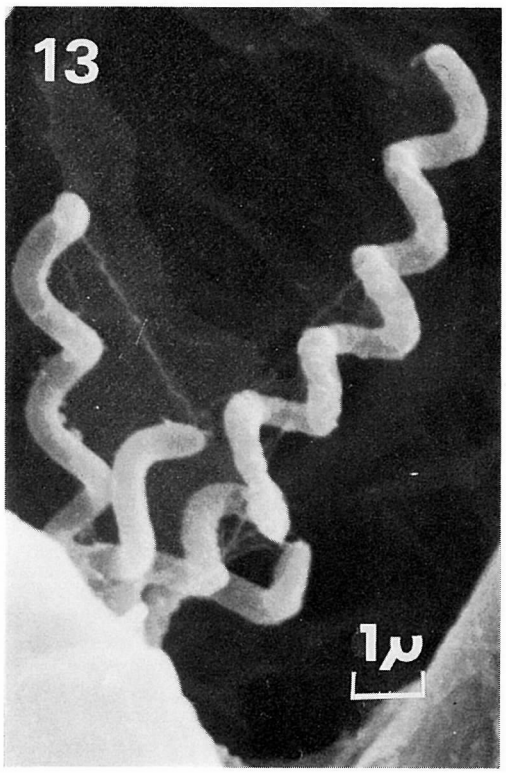

Fig. 13

Fig. 1 Marine spirillum. Spiral-direction unclear. Mounted on glass slide, air drying (AD). ×3, 300.

Fig. 2 Marine spirillum. Spiral-direction right-handed. Arrow a: Longer cell body with regular and complete turn, Arrow b: Shorter cell body with incomplete turn. Mounted on filter paper, critical point drying (CPD). ×11, 000.

Fig. 3 *Spirillum serpens.* Spiral-direction left-handed. Mounted on glass slide, CPD. ×7, 500.

Fig. 4 *S. serpens.* Spiral-direction right-handed. Mounted on filter paper, CDP. ×4, 500.

Fig. 5 *S. itersonii.* Spiral-direction left-handed. Mounted on filter paper, CPD. ×11, 500.

Fig. 6 *S. metamorphum.* Spiral-direction unclear. Mounted on glass slide, CPD. ×12, 000.

Fig. 7 *S. lunatum.* Spiral-direction unclear. Mounted on glass slide, CPD. ×28, 000.

Fig. 8 *S. psychrophilum.* Spiral-direction unclear. Mounted on filter paper, CPD. ×13, 000.

Fig. 9 *S. peregrinum.* Spiral-direction left-handed. Mounted on filter paper, CPD. ×11, 000.

Fig. 10 *S. hiroshimense.* Spiral-direction right-handed. Mounted on filter paper, CPD. ×5, 500.

Fig. 11 *S. multiglobuliferum.* Spiral-direction right-handed. Mounted on filter paper, CPD. ×25, 000.

Fig. 12 *S. petaginum.* Spiral-direction right-handed. Mounted on filter paper, CPD. ×11, 000.

Fig. 13 *Rhodospirillum rubrum.* Spiral-direction left-handed. Mounted on filter paper, CPD. ×9, 000.

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