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Leptospira sp. Strain Dimbovitza, First Isolate in Europe with Characteristics of the Proposed Genus *Leptonema*

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On the basis of serological, biological, and morphological characteristics of strain Dimbovitza cells, we concluded that this strain is a representative of the proposed genus *Leptonema* in the family *Leptospiraceae*.

Two leptospira-like spirochetes, illini strains 3055 and A177, have been isolated (7, 20). On the basis of their serological, biological, and morphological characteristics, these strains were recently proposed as representatives of a new genus, *Leptonema*, within the family *Leptospiraceae* (10).

During investigations on methods suitable for differentiating between pathogenic and saprophytic strains of *Leptospira*, we had the opportunity to examine a strain of another leptospira-like organism, strain Dimbovitza, which was isolated in Roumania in 1953. The characteristics of this strain were found to be similar in some respects to those of the two known illini strains. The results of a comparative study on strain Dimbovitza and illini strain 3055 are presented in this report.

MATERIALS AND METHODS

Strain Dimbovitza was originally isolated from the water of the Dimbovitza River in Roumania and was studied by Sefer et al. (16–19). This strain has been maintained in a modified Korthof medium (14).

Serology. Comparative serology studies were carried out with 27 serologically different strains of saprophytic leptospires and illini strain 3055 (Table 1). Furthermore, cells of strain Dimbovitza were tested with the 20 serogroup reference sera to pathogenic leptospires listed by Dikken and Kmety (6).

Biological characteristics. The following biological characteristics of strain Dimbovitza were determined: ability to grow in Trypticase soy broth (7), ability to grow in the presence of 225 μg of 8-azaguanine per ml (13) and at 13°C (12), and ability to induce hemolysis of sheep and rat blood cells (1, 5) and to hydrolyze phospholipids (2). The survival of the organisms in experimentally infected guinea pigs and golden hamsters was also examined (8).

Electron microscopy. Six-day-old cultures were centrifuged at 3,000 × *g* for 20 min, and the resulting pellet was suspended to a suitable density in SMC (0.03% sucrose in redistilled water supplemented with 0.01 M $MgCl_2$ and 0.01 M $CaCl_2$). Specimen grids were prepared from this suspension. Before staining, the cells on some grids were treated with drops of detergent (0.2% Teepol or 2% sodium deoxycholate, both in redistilled water). All preparations were negatively stained with 1% ammonium molybdate (pH 7) by the multiple drop technique (11).

Electron microscopy was carried out by using routine methods (11).

RESULTS

Serology. Cells of strain Dimbovitza showed no significant relationship to any of the saprophytic strains with which they were compared, including illini strain 3055 (Table 1). None of the 20 serogroup reference sera to pathogenic strains agglutinated cells of strain Dimbovitza (Table 1).

Biological characteristics. The biological characteristics of strain Dimbovitza were similar to those of illini strain 3055. Both strains grew in Trypticase soy broth, grew at 13°C, were resistant to 225 μg of 8-azaguanine per ml of medium, hemolyzed rat erythrocytes and lecithin, and survived for 4 h in guinea pigs and for 10 h in hamsters. Neither strain hydrolyzed sheep erythrocytes or sphingomyelin. Strain Dimbovitza and illini strain 3055 differed from the other saprophytic leptospires only by their ability to grow in Trypticase soy broth.

Electron microscopy. Cells of strain Dimbovitza showed a regular wavy outline and were 12 to 20 μm long and 0.1 μm wide. The wavelength was 0.5 μm. Each cell had two flagella, one inserted at each end (Fig. 1). In well-preserved cells each flagellum was entwined with the helical cell body for about six turns of the helix.

Cytoplasmic tubules with a diameter of about 8 nm were observed in cells accidentally damaged during preparation for electron microscopy (Fig. 2), as well as in cells treated with sodium deoxycholate. The cytoplasmic tubules appeared in bundles containing three to four tubules. The length of the tubules was roughly the same as the length of the flagella. One end of the bundle of tubules was frequently observed in close proximity to the insertion of the flagellum.

Flagella were detached from the cells after treatment with Teepol. The ultrastructures of the free flagella and their insertion regions were

TABLE 1. Results of comparative agglutination tests with saprophytic strains of *Leptospiraceae* and illini strain 3055

Immune serum to strain:	Homologous titer	Titer with strain Dimbovitza[a]	Strain Dimbovitza immune serum titer with strain listed[b]
illini 3055	12,800		
Veldrat S173	12,800		
Patoc 1	12,800		
Sao Paulo	50,000		
Andaman CH11	50,000		
Bovedo	12,800		
Cau	12,800		
Doberdo 1	12,800		
RPE	6,400		
AM-20	25,000		
Percedol 1	12,800		
Bazovizza	6,400		
S. Giusto	6,400		
Farneti	25,000	100	
Aurisina	25,000	100	800
Botanica	12,800		
Monte Fiascone 2	12,800		
Khoshamian	12,800		
Holland WaZ	25,000		
AM-6	6,400		
AM-13	12,800		
AR-18	12,800		
Friuli 37	6,400		
Friuli 44	12,800		
Friuli 48	12,800		
V-10	6,400		
V-1	25,000		
V-15	6,400		

[a] Titers are reciprocal dilutions of the sera.
[b] The strain Dimbovitza homologous titer was 25,000.

identical to those of flagella isolated from leptospires (3, 14); i.e., a hook and a basal knob consisting of two pairs of disks connected by a rod could be distinguished (Fig. 3).

DISCUSSION

Cells of strain Dimbovitza are morphologically similar to cells of illini strains 3055 and A177. However, it is interesting that these strains differ in the structure of the basal complex of the flagella; i.e., in strain Dimbovitza cells the basal complexes are similar to those of leptospires and other gram-negative bacteria, whereas the corresponding structures on flagella of illini strains 3055 and A177 are similar to the structures in treponemes, borreliae, and gram-positive bacteria. Cytoplasmic tubules were observed in the cells of strain Dimbovitza, and the presence of these tubules is considered to be a morphological character of cells of *Treponema* and of the illini strains (9, 10).

The biological properties of illini strain 3055 and strain Dimbovitza indicate some similarity between these strains and the majority of the biflexa strains of *Leptospira*. There is also some resemblance concerning pathogenicity; the short survival time of the cells of the illini strains in guinea pigs and hamsters is noteworthy.

From a practical point of view, we emphasize that the illini strains grow well in Trypticase soy broth. This can be used as an important and practical test for the differentiation of these strains from strains of *Leptospira biflexa*.

Strain Dimbovitza is serologically different from illini strain 3055, and for this reason the proposed genus *Leptonema* must comprise at least two serotypes, *illini* and *dimbovitza*. We

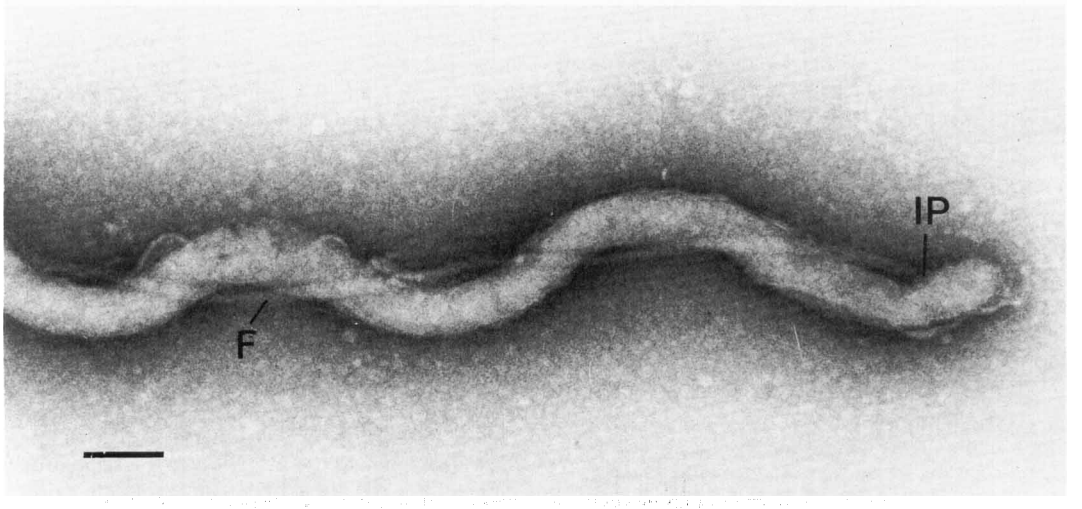

FIG. 1. End of a well-preserved strain Dimbovitza cell negatively stained with 1% ammonium molybdate (pH 7). The flagellum (F) is entwined with the cell body. IP, Insertion point of the flagellum. Bar = 100 nm.

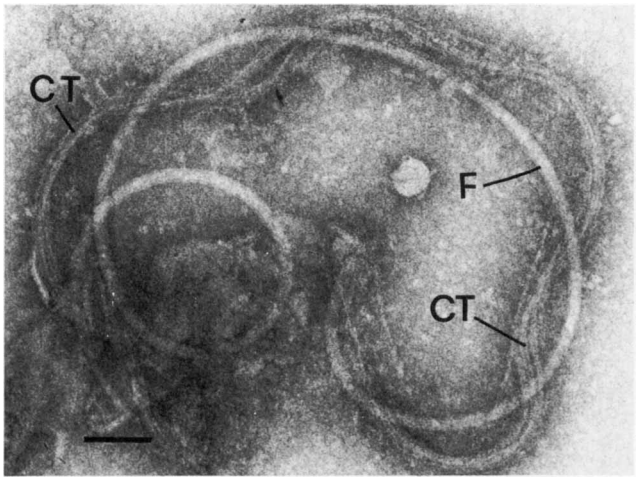

FIG. 2. End of a strain Dimbovitza cell accidently damaged during preparation for electron microscopy. The cell was negatively stained with 1% ammonium molybdate (pH 7). A bundle of four or five cytoplasmic tubules (CT) is apparent. F, Flagellum. Bar = 100 nm.

anticipate that other strains of this genus will appear in the future.

The guanine-plus-cytosine contents of the deoxyribonucleic acids of cells of the species of *Leptospira* that have been examined are 35 to 41 mol% (4), whereas the guanine-plus-cytosine contents of the deoxyribonucleic acids of the illini strains are 51 to 53 mol% (7). Thus determination of the guanine-plus-cytosine content of the deoxyribonucleic acid of cells of strain Dimbovitza will be of importance in confirming the relationship of this strain to the illini strains.

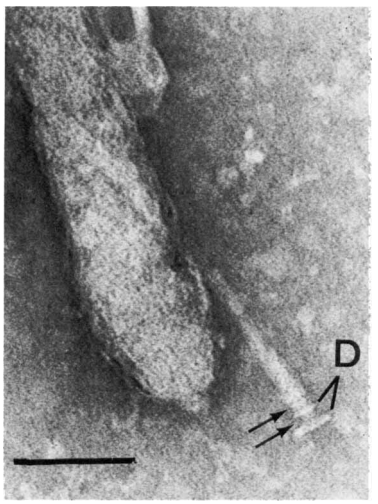

FIG. 3. Insertion part of a flagellum released from a strain Dimbovitza cell treated with Teepol and negatively stained with 1% ammonium molybdate (pH 7). The basal complex consists of two pairs of disks (D) connected to each other and to the hook by two rods (arrows). Bar = 100 nm.

Except for the isolation of two strains, illini strains 3055 and A177 (20), no information about the occurrence of strains of the proposed genus *Leptonema* has hitherto been published. Thus, strain Dimbovitza is the first known isolate of the proposed genus *Leptonema* from outside the United States.

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LITERATURE CITED

1. **Bazovská, S.** 1977. Hemolýza potkanich erytrocytov patogénnymi a saprofytárnymi kmeňmi leptospir. Cesk. Epidemiol. Mikrobiol. Immunol. **26**:275–283.
2. **Bazovská, S.** 1978. Sfingomyelinázova aktivita leptospirovych kultúr. Cesk. Epidemiol. Mikrobiol. Immunol. **27**:137–143.
3. **Birch-Andersen, A., K. Hovind-Hougen, and C. Borg-Petersen.** 1973. Electron microscopy of leptospira. I. *Leptospira* strain Pomona. Acta Pathol. Microbiol. Scand. Sect. B **81**:665–676.
4. **Brendle, J. J., M. Rogul, and A. D. Alexander.** 1974. Deoxyribonucleic acid hybridization among selected leptospiral serotypes. Int. J. Syst. Bacteriol. **24**:204–214.
5. **Chorváth, B.** 1975. Some properties of leptospiral hemolysin. Biológia (Bratislava) **30**:460–465.
6. **Dikken, H., and E. Kmety.** 1978. Serological typing methods of leptospires, p. 259–312. *In* T. Bergan and J. R. Norris (ed.), Methods in microbiology, vol. 11. Academic Press, Inc., London.
7. **Hanson, L. E., D. N. Tripathy, L. B. Evans, and A. D. Alexander.** 1974. An unusual leptospira, serotype *illini* (a new serotype). Int. J. Syst. Bacteriol. **24**:355–357.
8. **Hlavatá, Z., and S. Bazovská.** 1974. The possibility of differentiation of pathogenic and saprophytic strains of leptospires by their survival in organisms of laboratory

animals. Folia Fac. Med. Univ. Comenianae Bratisl. **12**(Suppl.):163–169.

9. **Hovind-Hougen, K.** 1976. Determination by means of electron microscopy of morphological criteria of value for classification of some spirochetes, in particular treponemes. Acta Pathol. Microbiol. Scand. Sect. B Suppl. **255**:20–30.
10. **Hovind-Hougen, K.** 1979. *Leptospiraceae*, a new family to include *Leptospira* Noguchi 1917 and *Leptonema* gen. nov. Int. J. Syst. Bacteriol. **29**:245–251.
11. **Hovind-Hougen, K., and A. Birch-Andersen.** 1971. Electron microscopy of endoflagella and microtubules in *Treponema* Reiter. Acta Pathol. Microbiol. Scand. Sect. B **79**:37–50.
12. **Johnson, R. C., and V. Harris.** 1967. Differentiation of pathogenic and saprophytic leptospires. I. Growth at low temperatures. J. Bacteriol. **94**:27–31.
13. **Johnson, R. C., and P. Rogers.** 1964. Differentiation of pathogenic and saprophytic leptospires with 8-azaguanine. J. Bacteriol. **88**:1618–1623.
14. **Kmety, E.** 1957. Návrh na štandartnú metódu aglutinolyzinovej reakcie pri leptospirózach. Cesk. Epidemiol. Mikrobiol. Immunol. **6**:372–377.
15. **Nauman, R. K., S. C. Holt, and C. D. Cox.** 1969. Purification, ultrastructure and composition of axial filaments from *Leptospira*. J. Bacteriol. **98**:264–280.
16. **Sefer, M.** 1965. Contributions à l'étude de la constitution chimique de la structure antigénique et du pouvoir pathogène des leptospires. Arch. Roum. Pathol. Exp. Microbiol. **24**:583–598.
17. **Sefer, M.** 1975. Ecological investigations on acvicola leptospira isolated in Romania, p. 49–55. *In* M. Sefer (ed.), Leptospirosis, leptospira and other spirocheta. Cantacuzino Institute, Bucharest.
18. **Sefer, M.** 1975. Growth and multiplication specific serum inhibition test applied to acvicola leptospira, p. 77–83. *In* M. Sefer (ed.), Leptospirosis, leptospira and other spirocheta. Cantacuzino Institute, Bucharest.
19. **Sefer, M., C. Verenca, and C. Sandru.** 1973. Metoda competitiei pentru controlul si purificarea culturilor de leptospire acvicole. Microbiol. Parazitol. Epidemiol. **18**:469–474.
20. **Tripathy, D. N., and L. E. Hanson.** 1973. Studies of *Leptospira illini*, strain 3055: immunologic and serologic determinations. Am. J. Vet. Res. **34**:563–565.