Description and Taxonomic Status of *Cytophaga heparina* (Payza and Korn) comb. nov. (Basionym: *Flavobacterium heparinum* Payza and Korn 1956)

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Flavobacterium heparinum was inadequately characterized when it was originally described by Payza and Korn in 1956. As a result it was listed as a species incertae sedis in the 8th edition of *Bergey's Manual*. The authors isolated a single strain of this organism, and presumably it was this strain which was deposited in the American Type Culture Collection (ATCC) under the number 13125 (= National Collection of Industrial Bacteria [NCIB] 9290). Because the original description was based on a single strain, ATCC 13125 is here regarded as the type strain of *F. heparinum* by monotypy. The results of an extensive study of the morphological, physiological, biochemical, and antimicrobial (lytic) properties of this strain suggest that it is more properly classified in the genus *Cytophaga* as *C. heparina* (Payza and Korn) comb. nov.

Very little is known about the organism described in 1956 by Payza and Korn as *Flavobacterium heparinum* (6, 7) beyond its ability to degrade heparin, which it can use as a sole carbon and nitrogen source (9). The organism's enzymes have been useful in the study of the structure of heparin (3). The study reported here was undertaken to update the description of this organism and to determine its proper taxonomic niche.

MATERIALS AND METHODS

Bacterial strains. A culture of American Type Culture Collection (ATCC) strain 13125 (= National Collection of Industrial Bacteria [NCIB] 9290) of *Flavobacterium heparinum* was obtained from NCIB. The control organisms used were obtained from NCIB, ATCC, and F.D. Cook (1, 2).

Methods. The methods used have been described previously (1, 2).

RESULTS

The characteristics of strain NCIB 9290 were found to be as follows.

Cell morphology. Gram-negative, nonflagellated, flexible rods (Fig. 1), 0.3 by 1.0 to 9.0 μm , showing gliding motility and limited ability to spread on certain media.

Cultural characteristics. On Cook cytophaga agar, 5-day-old colonies were deep yellow-gray (Munsell color: 2.5 Y 7/4), irregular, smooth to rough, lobate, umbonate, and transparent with no water-soluble pigment.

On skim milk-acetate agar, 5-day-old colonies were yellowish-orange-gray (Munsell color: 10 YR 7/4), irregular, smooth to rough, lobate, umbonate, and translucent with no water-soluble pigment.

On plate count agar, 5-day-old colonies were yellowish-orange-gray (Munsell color: 10 YR 6/4), more or less circular, smooth, entire, umbonate, and translucent with no water-soluble pigment.

The Munsell color of colonies on various media ranges from 10 YR 6-7/4-6 and also 2.5 YR 7/4; no water-soluble pigment has ever been observed.

In overnight-shaken broth culture, the growth is slightly silky, but not viscous or flocculent; no surface ring or pellicle is produced.

Neither fruiting bodies nor microcysts are produced.

Physiology. Growth was not reduced by up to 3% NaCl; the preferred atmosphere was air or 10% O₂; the temperature range was 10 to 30°C; optimum growth occurred at 20 to 35°C; the pH range was 7 to 10; optimum growth also occurred at pH 7 to 10.

Utilizes NH₄⁺, urea, asparaginate, and some proteins and protein digests (e.g., tryptone, casein), but not NO₃⁻, glutamate, or certain other proteins and protein digests (e.g., gelatin, milk, Casitone) as sole N sources.

Growth was stimulated by yeast extract.

Growth was reduced by 0.01% and inhibited by 0.1% sodium lauryl sulfate. Susceptible to 300 U of polymyxin B and to actinomycin D. Resistant to 30 μg of chloramphenicol, 10 μg of dihydrostreptomycin, and to 10 U of penicillin.

Except for one actinomycete (UASM 4432) and possibly another (UASM 4441), bacteria and fungi were not lysed; a chlorella was also possibly lysed.

Biochemical reactions. Attack on glucose was both oxidative and slowly fermentative.

FIG. 1. Cells of strain NCIB 9290.

Acid was produced from cellobiose, sucrose, lactose, glucose, mannitol, and glycerol (slow).

Lipolytic on Tweens 20, 40, 60, and 80.

Starch, cellulose (filter paper or carboxymethyl cellulose), chitin, agar, pectate, and alginate were not hydrolyzed.

Gelatin was not liquefied and milk was not peptonized; growth occurred in Casitone broth; growth and NH_3 were produced from casein and slowly from Casamino broths. The organism grew on 0.2% tryptone agar and hemolyzed sheep erythrocytes in 7 but not in 2 days.

H_2S , catalase, oxidase, and phosphatase were produced; the indole-methyl red-Voges-Proskauer-citrate tests were negative; no growth occurred on McConkey or eosin methylene blue agar. Did not reduce NO_3^- or NO_2^- to gaseous form. Deoxyribonuclease was not produced.

DISCUSSION

The name *Flavobacterium heparinum* Payza and Korn is validly published and legitimate (4, 9), but since so little was known about this organism, Weeks relegated it to the species incertae sedis section of *Flavobacterium* in the 8th edition of *Bergey's Manual* (9). There is no indication that Payza and Korn isolated more than one strain of *F. heparinum*, and it is assumed that the organism deposited with the ATCC by Korn under the number 13125 (= NCIB 9290) is the isolate on which the original description of *F. heparinum* was based. The identification of this strain has been verified by the ATCC, and this strain is here regarded as the type strain of *Flavobacterium heparinum* by monotypy. ATCC 13125 was used by Dietrich (3) for studies of heparin structure, and it is the strain used in the present study, having been obtained from NCIB as 9290. The organism has been shown to glide (5, 8), and it degrades heparin, a mucopolysaccharide acid composed of

hexosamine and hexuronic acid units containing sulfuric acid ester groups. Heparin has the properties of a polymer and is an anticoagulant produced by mast and liver cells. Nonfruiting gliding organisms which have a low guanine plus cytosine (G+C) content in their deoxyribonucleic acids and which degrade polysaccharides are placed in the genus *Cytophaga* (5) of the order *Cytophagales*. This investigation confirms the gliding motility of and presents further data on the organism, none of which specifically excludes it from the genus *Cytophaga*.

However the G+C value of this organism, which has recently been revised upwards to 45.6 mol% (8) from its originally quoted value of 42.2 mol% (5), poses a problem. The range of the G+C contents of species accepted into the genus *Cytophaga* in the latest edition of *Bergey's Manual* (9) is 33 to 42 mol%. NCIB 9290 is clearly outside this already wide range for one genus, but if one then considers the G+C contents of the deoxyribonucleic acids of other polysaccharolytic species which also seem to belong to *Cytophaga* (Table 1), then the range is extended to 32.5 to 48.7 mol%. It is fairly obvious that this "*Cytophaga* group" is still a heterogeneous mix-

TABLE 1. Known G+C contents of the deoxyribonucleic acids of polysaccharolytic cytophagas and putative cytophagas

Organisms	G+C ^c content (mol%)
Marine species	
Agarolytic	
<i>Cytophaga lytica</i>	33.2-34.2
<i>C. latercula</i>	34.2
<i>C. diffluens</i>	34.7-42.3
Amylolytic	
<i>C. fermentans</i>	39
Cellulolytic ^a	
<i>Microscilla arenaria</i>	32.5
<i>M. aggregans</i>	36.5-39.5
<i>M. sericea</i>	38-39
<i>M. furvescens</i>	44
Soil and freshwater species	
Chitinolytic	
<i>C. johnsonae</i> ^b	32.7-35
<i>Flexibacter</i> sp. FS-1	48.7
Agarolytic	
<i>C. stevensis</i>	34.9
Cellulolytic ^a	
<i>C. hutchinsonii</i>	39
<i>F. flexilis</i> subsp. <i>pelliculosus</i>	39.5
<i>C. aurantiaca</i>	42
<i>F. sancti</i>	46-47

^a Degradation of filter paper or carboxymethyl cellulose or both.

^b Includes *Flavobacterium pectinovorum* (1).

ture of organisms. When other organisms are described and more comparative studies are done, a basis for intelligent subdivision of the group may become apparent.

At present there is still an inadequate separation of *Cytophaga* from *Flexibacter*, which has a similar, wide G+C range (29.8 to 47.5 mol%). It is the author's opinion that there are not enough data on which to subdivide either of these genera any further. Those forms having any polysaccharase at all should be assigned to the genus *Cytophaga* until our knowledge permits the eventual redistribution of this group of gliding, nonsheathed, and nonhelical organisms into better defined, more restricted genera. Hence, it is suggested that the organism described here be removed from the genus *Flavobacterium* and be assigned to the genus *Cytophaga* as *Cytophaga heparina* (Payza and Korn) comb. nov. because it differs significantly from any of the presently described *Cytophaga* species.

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