THE CYTOPHAGA-LIKE BACTERIA: A SEARCH FOR KEY CHARACTERS

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Summary

Nearly 800 Cytophaga-like bacteria (CLB) were isolated from soil and freshwater. Mainly out of these isolates, 185 strains were chosen for a phenotypic analysis, employing 130 different tests. The aim was to find specific traits that would lead to a reasonable clustering and thus could be used as taxonomical key characters. An apparently reasonable classification was obtained with the following characters: gliding motility,cell morphology, cellular shape-change, presence of flexirubin-type pigments and of certain enzymes (catalase, cytochrome oxidase, lecithinase, urease, phenylalanine deaminase, DNase, starch hydrolysis, and gelatin liquefaction). The characters were weighted and used in the order given above. Obviously, this chosen hierarchy has an influence on the resulting arrangement of the strains. Nonetheless, it was possible to define certain groups quite reliably, such as Flexibacter and Lysobacter. However with other groups, particularly with subgroups, results were often ambiguous. A comparison with data obtained by DNA/DNA hybridization /4/ suggests, that for the different groups and subgroups of CLB different sets of key characters have to be applied, or that at least the hierarchy of the characters has to be altered.

Anyone familiar with soil and freshwater Cytophaga-like bacteria (CLB) will agree, that the taxonomy of these microorganisms still needs intensive study. Much of the uncertainty in this field is due to the fact that in the past most investigators concentrated on physiologically or ecologically specialized groups of organisms, as cellulose, chitin, agar decomposers, fish pathogens etc. This prevented the development of a broad taxonomical concept for the CLB. More recently disagreement with definitions has also contributed to the problem. Although several extensive research reports have been published on this subject within the past decade /7, 8, 10, 11, 15, 17, 18, 21, 22, 23, 24, 25, 26/, we felt that a comprehensive phenomenological study on a large number of diverse and newly isolated strains would be desirable. For neither was it clear to us, whether the existing taxa of CLB were realistic, nor whether their boundaries were well defined, nor even how many taxa there are. Furthermore, our hope was to find additional key characters, that could be used to identify CLB more reliably. As a high degree of uncertainty is connected with any classification based on morphological and physiological data alone, a parallel study was started on a selection of the same strains applying methods of molecular taxonomy /4/.

Gliding motility and yellow pigmentation, which are considered as essential characters of the CLB /20/, served as criteria for recognition. In preliminary experiments, using available strains of Cytophaga johnsonae and Flexibacter elegans, we ascertained which media would allow the expression of these characters. All samples were then plated on several of the differentiating media (details will be published elsewhere). Every colony type appearing on the plates was examined. All unicellular, non-flagellated, gram-negative organism demonstrating gliding, with or without yellow pigmentation, or which did not glide, but were pigmented, were then isolated and cloned.

Then, 185 strains were chosen for a phenotypic analysis. Of these, 120 were randomly selected from the above-mentioned collection, 28 strains had previously been isolated, 7 came from Dr. Güde /16/, the remaining 30 organisms were kindly supplied by other laboratories. Based on classical diagnostic procedures /3, 5, 12/, on the results of previous taxonomic studies on CLB /7, 9, 17, 22/, and on preliminary experiments that were performed with some randomly selected strains, 130 different tests were chosen to be performed on these 185 organisms. Morphological studies on different media were followed by physiological and biochemical characterization.

Two traits proved of paramount importance: cellular shape-change and presence of flexirubin-type pigments. A peculiar cellular shape-change has been described for two flexibacteria, i.e. for FS1 /29/ and for Fx el /28/. In young liquid cultures the cell population consists almost exclusively of very long thread-cells, up to 50 µm in length. These thread-cells are extremely active, gliding, and bending, flexing and twisting in a whip-like motion. As the cultures age, the thread-cells fragment and the cell population changes into short, often almost coccoidal, immotile rods, 2 to 8 µm long. A different cellular shape-change has been described for Cy. johnsonae Cy j1 /1/. Here, in young, actively growing cultures the cells are uniformely short, only 1.5 to 2.5 µm long. As the culture reaches the stationary growth phase, the cells progressively elongate up to 40 µm and more. The long cells are, however, not agile, and have a rough, somewhat broken appearance. These two forms of cellular shape-change were noticed in the present investigation only in peptone containing media. Flexirubin-type pigments can be easily detected by covering the colonies with KOH solution: their yellow or orange color changes then into purple or brown (= KOH-positive) /28/.

The ability to move by gliding was ascertained in chamber cultures, on peptone agar, in all cases, and 92 % of all tested organisms were found positive. Of these gliding strains, again nearly 90 % displayed a dimorphism as described for Cy j1 (called here shape-change 1; Fig. 1). Another 10 % of the gliders, 15 strains, displayed the dimorphism typical for Fx e1 (= shape-change 2; Fig. 1). With the remaining gliders, no definite cellular shape-change could be observed under our experimental conditions (= shape-change 0). Nor did the 8 % of unicellular, non-flagellated, non-gliding organisms show any form of cellular shape-change.

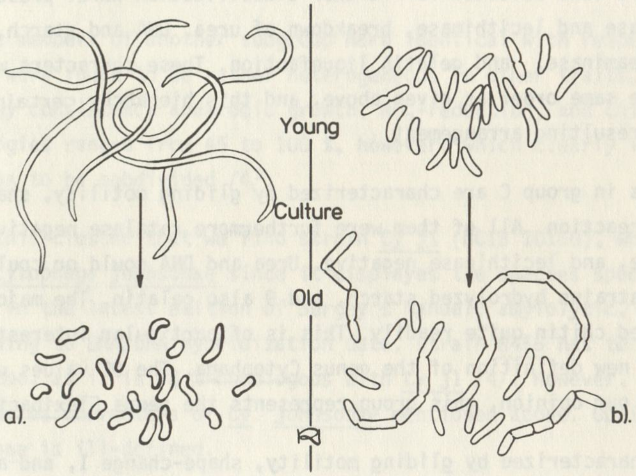
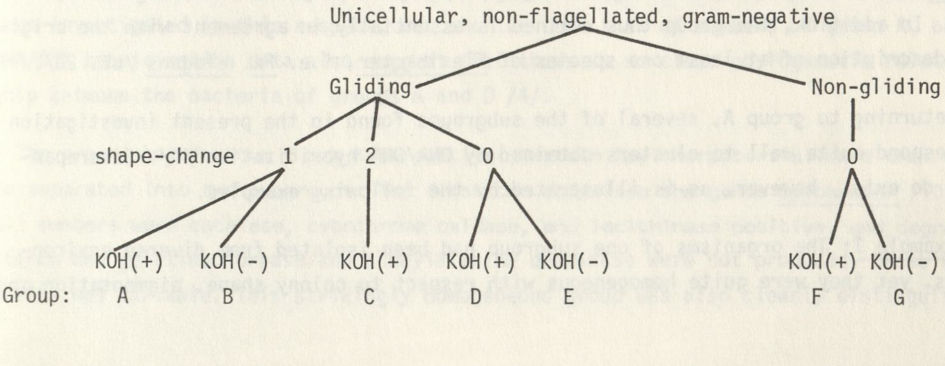


Fig. 1. a) Shape change 2; b) shape change 1.

Approximately 80 % of all tested strains were KOH-positive, which was taken to indicate presence of flexirubin-type pigments. Of these KOH-positive bacteria 95 % simultaneously displayed a cellular shape-change either of type 1 or of type 2. KOH-positive strains were also found among gliders without dimorphism and among non-gliding organisms. KOH-negative strains were noted among gliding as well as among non-gliding bacteria, both types always without a cellular shape-change, however. The scheme below depicts the main groups obtained when the three characters: gliding motility, cellular shape-change, and KOH-reaction are applied to classification.



In order to obtain subgroups we then looked for stable morphological and biochemical characters that showed a high degree of correlation. For example, one group of strains formed pale-yellow colonies with smooth, transparent swarm-edges on a certain type of solid medium. On another agar substrate the colonies would be bright yellow and surrounded by a brown zone, due to a water-soluble pigment diffusing into the medium. The same bacteria displayed all the same cellular shape-change and KOH-reaction and could always degrade urea.

The characters thus selected for further classification were: presence of catalase, cytochrome oxidase and lecithinase, breakdown of urea, DNA and starch, production of phenylalanine deaminase, and gelatin liquefaction. These characters were applied in all cases in the same order as given above, and this hierarchy certainly has an influence on the resulting arrangement.

The organisms in group C are characterized by gliding motility, shape-change 2 and a positive KOH-reaction. All of them were furthermore catalase negative, cytochrome oxidase positive, and lecithinase negative. Urea and DNA could or could not be degraded. All 15 strains hydrolyzed starch, and 9 also gelatin. The majority of these bacteria degraded chitin quite readily. This is of particular interest with respect to the proposed new definition of the genus Cytophaga. The GC values were between 44 to 51 % /4/. In our opinion, this group represents the genus Flexibacter /28/.

Group A is characterized by gliding motility, shape-change 1, and a positive KOH-reaction. Here we find 67 % of all organisms tested. Morphologically, these bacteria were slender rods with round or slightly tapered ends. Pigmentation went from pale yellow to deep orange. The majority of the strains was catalase positive, oxidase and lecithinase negative. Each strain degraded at least one kind of complex polysaccharide (chitin, CMC, starch etc.), but none was able to decompose cellulose. The GC values of those strains that have been investigated fell within the range of 32 to 37 % /4/. These organisms we consider as the cytophagas s. str. They can reliably be separated and distinguished from the flexibacters (group B). There is no overlap of the two GC ranges, which gives additional support on the molecular level to the idea that we are dealing with two distinct groups of organisms. We feel, that the differentiating characters mentioned above are better suited to separate the genus Flexibacter from the cytophagas than the single property of polysaccharide degradation /20/. In addition, the group thus defined is essentially in agreement with the original description of at least one species of Flexibacter, i.e. Fx. elegans /cf. 28/.

Returning to group A, several of the subgroups found in the present investigation correspond quite well to clusters obtained by DNA/DNA hybridization /4/. Discrepancies do exist, however, as is illustrated by the following examples.

Example 1: The organisms of one subgroup had been isolated from diverse environments, yet they were quite homogeneous with respect to colony shape, pigmentation and several other key characters. They were the only representatives of group A that were able to degrade urea. Furthermore, all were facultative anaerobes and utilized xylose. DNA/DNA hybridization studies revealed, that one strain, Cy 460, did not belong in this subgroup, for it showed only 40 % homology with the other members, which among themselves were 100 % homologous /4/. Strain Cy 460 had been placed in this subgroup mainly on the basis of urea decomposition and anaerobiosis. Its colony form and pigmentation differed slightly from the remaining representatives of the group.

Example 2: The members of another subgroup were identical with respect to the key characters, but were found to be rather heterogeneous in other traits, such as pigmentation, colony consistency anaerobic growth, NO_3-reduction, and chitin degradation. DNA homologies ranged from 45 to 100 %, however, which clearly indicates that this subgroup has to be subdivided /4/.

It is within this cluster that we find strain Cy j1 (NCIB 10150), which is considered a typical Cytophaga johnsonae since it displayes the species specific properties, as stated in the latest edition of Bergey's Manual: amylolytic, chitin-degrader /20/. According to DNA/DNA hybridization data, strain H456 has to be regarded as Cy. johnsonae, too, as it is 70 % homologous with Cy j1 /4/. However, H456 is negative for the species characters of Cy. johnsonae mentioned above. Obviously, the species Cy. johnsonae is ill-defined.

Group B consists of only one strain, Cy. flevensis Cy f1 (= A-34), an agar-decomposer isolated from the IJsselmeer, an old North Sea bay /34/. This organism does not contain flexirubin-type pigments, which is typical for marine CLB, and we feel that Cy f1 is actually a marine organism.

Groups D and E contain strains that glide, but do not display a cellular shape-change. All strains of group D came from heavily polluted environments. Their rod-cells were generally rather coarse and somewhat pleomorphic. In several of their characters these strains corresponded with the cytophagas of group A. They produced catalase and cytochrome oxidase, and were KOH-positive. The majority were lecithinase positive, which is in contrast to the usual soil and freshwater cytophagas. The GC-values were between 32 and 36 % /4/. However, the genome length of four strains that have been investigated in this respect, was shorter than that of members of group A, and DNA/DNA hybridization data also suggest that there is no close phylogenetic relationship between the bacteria of groups A and D /4/.

The organisms of group E are KOH-negative. Certain key characters allowed them to be separated into two subgroups. The first one comprises the genus Lysobacter /10/. All members were catalase, cytochrome oxidase, and lecithinase positive, and degraded starch and gelatine. Urease and phenylalanine deaminase were not produced. DNase activity was variable. This strikingly homogeneous group was also clearly distinguishable by the very mucoid colonies, and the frequent production of water soluble pigments. Their isolated position was verified by high GC-values between 66 and 70 % /4/. The members of the second subgroup of group E corresponded in several key characters to certain strains of group A (catalase (+), cytochrome oxidase (-), lecithinase (-), ability to degrade starch and gelatine), but differed from the cytophagas in colony form and pigmentation. Their colonies often showed a greenish hue, and generally failed to spread. Unfortunately, only few GC and no hybridization data are presently available for this subcluster. The GC-content of one strain, Cy H3, was 41 % /4/, i.e. slightly higher than with the cytophagas of group A.

The last two groups, F and G, comprise non-gliding organisms. None of the 13 strains showed a cellular shape-change. Approximately 50 % contained flexirubin-type pigments (group F). The majority of these non-gliding organisms resembled the cytophagas in their key characters. However, they generally required growth factors, and their ability to degrade polysaccharides, except for starch, was practically nil. Nine of these strains came from Dr. Hayes' collection of low GC flavobacteria /17/. In fact, overlapping GC-values, common presence of flexirubin-type pigments, similarities in the fatty acid composition /13/, as well as in the quinone spectra /6/ indicate a phylogenetic relationship between certain flavobacteria and the CLB.

When we attempted to place our new isolates into existing taxa of CLB, we were confronted with difficulties. The reasons for this situation are that 1) original type strains, that would allow a direct comparison, are no longer available; 2) the descriptions of the species are often incomplete and vague, resting sometimes solely on differences in temperature optima or colony pigmentation; 3) there is often a lack of adequate comparative studies, so that the boundaries of the species cannot be recognized. The existing "species" must thus be regarded as very problematical. Still another reason for our troubles emerged, however, from our own studies /cf. also 4/: It turned out that the group of the freshwater and soil CLB is far more complex than has previously been assumed. We feel that we should be careful about assigning specific names to any of our isolates.

The data presented in this article are taken from Hirsch, I.: Beiträge zur Taxonomie der Cytophagales auf Grund morphologischer und stoffwechselphysiologischer Eigenschaften. Doktor Diss., TU Braunschweig, FRG (1980), 142 pp.

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