Emendation of *Xanthobacter flavus* as a Motile Species

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Xanthobacter flavus 301^T (T = type strain) and other strains, including H4-14, both of which were previously described as nonmotile, were reproducibly motile and peritrichously flagellated during the log phase when they were cultured in medium lacking tricarboxylic acid cycle intermediates. Therefore, the species description is emended to include motility and flagellation. Similarly, *Xanthobacter autotrophicus* was found to be flagellated and motile, but this finding was not consistently reproducible and was mainly found with the mutant strain Fe^{-s}.

Currently, the genus *Xanthobacter* (10) consists of three species, *Xanthobacter autotrophicus* (the type species), *Xanthobacter flavus*, and *Xanthobacter agilis*. Strains of *X. autotrophicus* (11) and *X. flavus* (3, 5) have been described as being nonmotile, whereas strains of *X. agilis* have been described as being motile (2). *X. flavus* is the only biotin-requiring species. Typically, new *Xanthobacter* isolates are classified tentatively into one of the three *Xanthobacter* species on the basis of motility and biotin requirement (12). Recently, Reding et al. reported that *Xanthobacter* strains isolated from the roots of rice were motile but differed morphologically and physiologically from strains of *X. agilis* (7, 8). Meijer et al. have described a *Xanthobacter* strain, strain 25a, that is motile and requires biotin (6).

During routine growth experiments, we observed that *X. flavus* was motile under certain culture conditions. Motility depended on the growth phase and the carbon source (Table 1). In general, growth in the presence of tricarboxylic acid cycle intermediates produced nonmotile cells, whereas growth on alcohols or transfer of washed cells to medium lacking tricarboxylic acid cycle intermediates resulted in motile cells. Growth on H₂-CO₂, glutamate, or glutamine also produced nonmotile cells. However, we emphasize the effect of tricarboxylic acid cycle intermediates because

growth on these compounds produces swollen, pleomorphic cells in *X. autotrophicus* and *X. flavus* but scarcely in *X. agilis*. This characteristic has been used to differentiate *Xanthobacter* species (10). Cells of *X. flavus* produce large amounts of poly-β-hydroxybutyrate as a carbon reserve (10), and we assume that this compound serves as a carbon and energy source for flagellum synthesis and motility when cells are incubated in a medium lacking usable carbon. The nitrogen source used (NH₃, NO₃, or N₂) had no effect on motility.

When cultures were grown with 0.1% *n*-propanol as the sole carbon source, approximately 20% of the *X. flavus* H4-14 cells and 35% of the *X. flavus* 301^T (= DSM 338^T) (T

TABLE 1. Effect of carbon source on the motility of *X. flavus* and *X. agilis*^a

Substrates	Motility of:	
	<i>X. agilis</i> SA35	<i>X. flavus</i> 301 ^T
Citrate, fumarate, malate, succinate, glutamate, glutamine, yeast extract, H ₂ -CO ₂	+	–
Methanol, ethanol, <i>n</i> -propanol, isopropanol, butanol, gluconate	+	+

^a Cultures were grown to late log phase and transferred (2%, vol/vol) to test medium containing a carbon source at a concentration of 0.2% (wt/vol). Motility was determined by examining wet mounts with a Vanox phase-contrast light microscope (Olympus, Hyde Park, Pa.). Only the substrates which supported growth of both species were used for comparison. +, cells motile in the presence of the substrate; –, cells not motile in the presence of the substrate.

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FIG. 1. *X. flavus* H4-14, showing flagellum insertions (arrows). (Inset) Magnified view of the region in brackets, showing flagellum insertion. Samples were negatively stained by using the single-carbon-layer technique of Valentine et al. (9) and were viewed by using a JEOL model 100CXII transmission electron microscope.

FIG. 2. (A) *X. flavus* 301^T cell with two coiled flagella. (Inset) Magnified view of the region in brackets, showing insertion of one of the flagella. (B) Isolated flagellum of *X. flavus* 301^T forming a wavelength pattern. For the methods which we used, see the legend to Fig. 1.

= type strain) cells were motile throughout the log phase by means of retarded peritrichous flagella (Fig. 1 and 2). Motile cells of *X. flavus* 25a, SA12, and MA10 (3) were also observed during growth on propanol; however, no motile

FIG. 3. *X. agilis* SA35^T cell with four peritrichous flagella. For the methods which we used see the legend to Fig. 1.

TABLE 2. Comparison of the flagella of *X. flavus* and *X. agilis*^a

Strain	No.	Diam (nm)	Length (μm) ^b	Wavelength (μm) ^c
<i>X. agilis</i> SA35 ^T	2-5	14.2	9.2	2.0 (0.26)
<i>X. flavus</i> 301 ^T	1-7	14.5	14.0	2.5 (0.37)
<i>X. flavus</i> H4-14	2-7	14.2	13.0	2.2 (0.7)

^a *X. flavus* 301^T and H4-14 cells were cultured on medium containing 0.1% *n*-propanol, and *X. agilis* SA35^T cells were cultured on medium containing 0.1% fumarate. Data for *X. autotrophicus* 7cSF are not given because of inconsistent flagellation. All cells with more than one flagellum were peritrichous. When a cell had only one flagellum, no specific placement of the flagellum was apparent.

^b Longest observed filament length.

^c Mean (standard deviation) of the wavelength, when it was observed.

cells were found in cultures of *X. flavus* SA14 and CB2 (3) when these organisms were cultured under similar conditions. Under culture conditions which resulted in nonmotile cells (e.g., growth in the presence of succinate), no cells possessed flagella.

We found that up to 50% of the cells of *X. autotrophicus* 7cSF (= DSM 2267) (a slime-reduced mutant of type strain 7c [= DSM 432]) were motile in the very early log phase when cultures were grown with 0.1% *n*-propanol as the sole carbon source in the absence of tricarboxylic acid cycle intermediates. However, for unknown reasons, production of motile cells was not always reproducible. *X. autotrophicus* 7c^T, JW33 (reference strain [= DSM 1618]), JW21, and JW4 were nonmotile and nonflagellated under all of the growth phase and carbon source conditions tested (data not shown). Nevertheless, it is possible that strains of *X. autotrophicus* may become motile if certain conditions exist, including a reduction in the amount of slime produced.

The motility of *X. agilis* was not dependent on the carbon source since all growing cultures contained motile cells (Table 1). *X. agilis* has retarded peritrichous flagella (1) (Fig. 3).

The flagellation of *X. flavus* and the flagellation of *X. agilis* were comparable with respect to number, position, diameter, length, and wavelength of the flagella (Table 2). Thus, the type of flagellation cannot be used as a taxonomic tool. However, the motility of *X. flavus* differs from the motility of *X. agilis* in that *X. agilis* is motile irrespective of the carbon source. In light of this, the presence or absence of motility should not be used any longer as a main criterion for assigning *Xanthobacter* isolates to one of the three *Xanthobacter* species.

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