



# Inactivation of ferric uptake regulator (Fur) attenuates *Helicobacter pylori* J99 motility by disturbing the flagellar motor switch

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## Abstract:

**Background:** Flagellar motility of *Helicobacter pylori* has been shown to be important for the bacteria to establish initial colonization. The ferric uptake regulator (Fur) is a global regulator that has been identified in *H. pylori* which is involved in the processes of iron uptake and establishing colonization. However, the role of Fur in *H. pylori* motility is still unclear.

**Materials and Methods:** Motility of the wild-type, *fur* mutant, and *fur* revertant J99 were determined by a soft-agar motility assay and direct video observation. The bacterial shape and flagellar structure were evaluated by transmission electron microscopy. Single bacterial motility and flagellar switching were observed by phase-contrast microscopy. Autoinducer-2 (AI-2) production in bacterial culture supernatant was analyzed by a bioluminescence assay.

**Results:** The *fur* mutant showed impaired motility in the soft agar assay compared with the wild-type J99 and *fur* revertant. The numbers and lengths of flagellar filaments on the *fur* mutant cells were similar to that of the wild-type and revertant cells. Phenotypic characterization showed similar swimming speed but reduction of switching rate in the *fur* mutant. The AI-2 production of the *fur* mutant was dramatically reduced compared with wild-type J99 in log phase culture medium.

**Conclusions:** These results indicate that Fur positively modulates *H. pylori* J99 motility through interfering with bacterial flagellar switching.

Fig. 1A

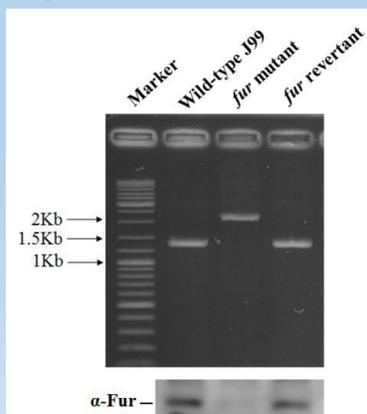
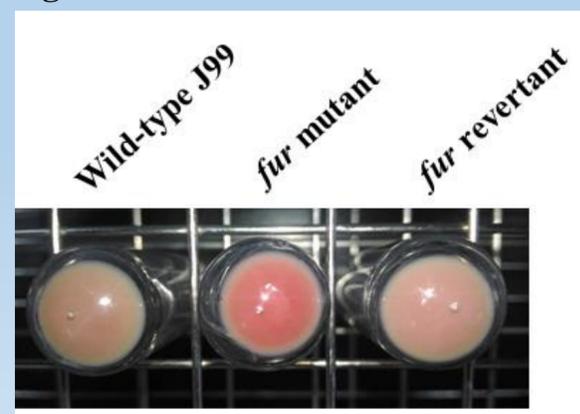


Fig. 1B



**Figure 1. Construction and confirmation of the *fur* mutant and *fur* revertant.** (A) Confirmation of the *fur* mutant J99 by PCR (upper panel) and western blotting (lower panel). The chromosomal DNA extracted from wild-type J99, *fur* mutant, and *fur* revertant served as template for PCR analysis. GeneRuler™ DNA Ladder Mix marker DNA was used (Fermentas). For western blotting analysis, lysates of the examined strains were extracted and probed with anti-Fur polyclonal antibody. (B) Iron uptake analysis of the wild-type J99, *fur* mutant, and *fur* revertant.

Fig. 2A

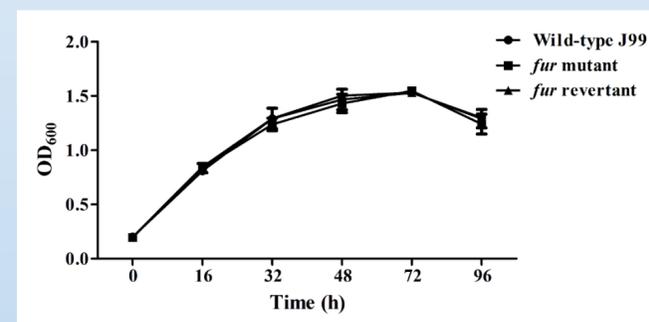
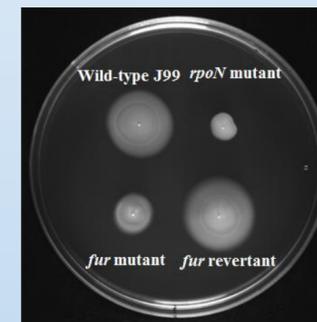


Fig. 2B



**Figure 2. Growth curve and motility of the wild-type J99, *fur* mutant, and *fur* revertant.** (A) The growth rate was determined at different time points. (B) The motility of the three examined strains was determined by soft agar plates. *rpoN* mutant was used as an internal control (non-motile strain).

Fig. 3A

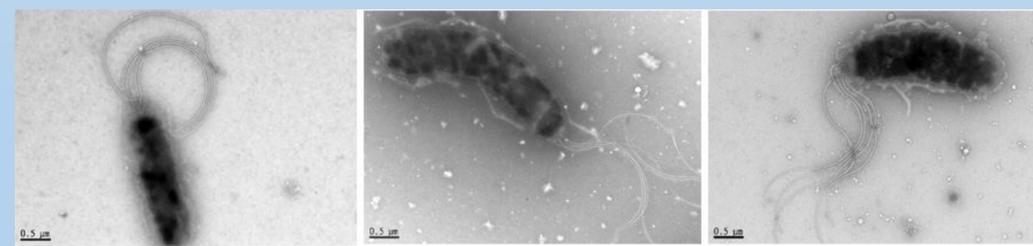
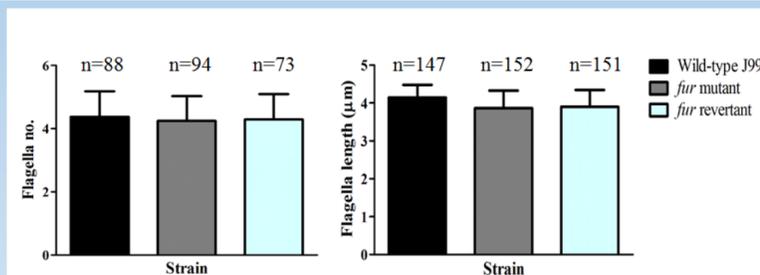


Fig. 3B

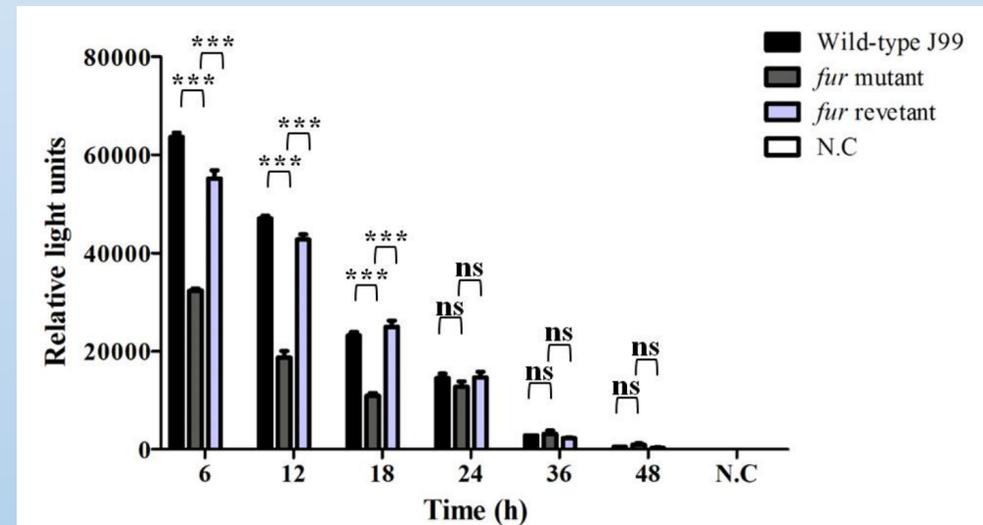


**Figure 3. Transmission electron micrographs of negatively stained *Helicobacter pylori*.** (A) Flagella structure of the wild-type (left), *fur* mutant (middle), and *fur* revertant (right). (B) Numbers and lengths of flagella on examined cells. Scale bars represent 0.5 μm.

Table 1. The average switching rate in different conditions.

Switching Rate (1/s)	Wild-type J99	<i>fur</i> mutant	<i>fur</i> revertant
Edge of soft agar	0.97	0.86	0.93
Center of soft agar	0.97	0.78	0.94

Fig. 4



**Figure 4. Extracellular AI-2 production of the wild-type J99, *fur* mutant, and *fur* revertant.** The production of extracellular AI-2 as measured by using a *V. harveyi* bioluminescence assay is shown in cultures of the wild type J99, *fur* mutant, and *fur* revertant cells grown to various time points.

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