

**Title**

*N*-glycosylation of a cargo protein C-terminal domain recognized by the type IX secretion system in *Cytophaga hutchinsonii* affects protein secretion and localization

**Running title**

Protein *N*-glycosylation in *Cytophaga hutchinsonii*

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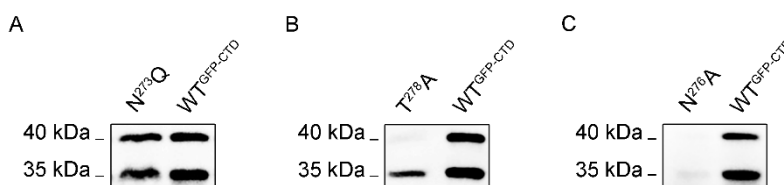
## Supplemental Material

**Fig. S1**



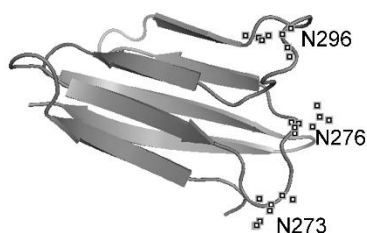
**Fig. S1** The modification of the GFP-CTD<sub>CHU\_2708</sub> fusion protein occurred in the periplasm. The periplasmic and cytoplasmic proteins (Cyt) of the WT<sup>GFP-CTD</sup> separated by SDS-PAGE, then identified by Western blotting with the anti-GFP antibody.

**Fig. S2**



**Fig. S2** Site-directed mutagenesis of CTD<sub>CHU\_2708</sub>. (A) The mutation of N273 in CTD<sub>CHU\_2708</sub> to glutamine. (B) The changing of T278 of CTD<sub>CHU\_2708</sub> to alanine. (C) The mutation of N276 of CTD<sub>CHU\_2708</sub> to alanine. The periplasmic space proteins of the WT<sup>GFP-CTD</sup>, N<sup>273</sup>Q, T<sup>278</sup>A, and N<sup>276</sup>A strains were identified by Western blotting with the anti-GFP antibody.

**Fig. S3**



**Fig. S3** The structural prediction of CTD<sub>CHU\_2708</sub>. The sites N273, N276, and N296

are shown.