CHARACTERIZING THE ROLES OF AGMT, A PUTATIVE LYTIC TRANSGLYCOLYASE, IN THE GLIDING MOTILITY OF *MYXOCOCCUS XANTHUS*

A thesis

By

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ABSTRACT

The gram-negative bacterium Myxcoccus xanthus glides on solid surfaces utilizing an inner-membrane proton channel as the motor. Together with other accessory proteins, motor units form static complexes that exert force between internal helical tracks and the substratum and drive a corkscrew-like motion of the tracks. As a result, the cell also moves forward like a corkscrew. After transient stalls, static complexes quickly disassemble and resume rapid motion. However, is not clear how the mechanical force transmits to cell surfaces across the rigid peptidoglycan (PG) cell wall. Here we show that AgmT, a putative lytic transglycosylase for PG, is an essential component of the gliding machinery. Using single-molecule microscopy, we found that the motors move normally in the absence of AgmT but fail to stall. Thus, we have identified the connection between the gliding motor and the PG. The aim of my research is to investigate how AgmT interacts with PG and if its transglycosylase activity is required for gliding. The findings of this project will reveal the mechanism by which M. xanthus transmits proton motive force from the inner-membrane to the cell surface.

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CONTRIBUTORS AND FUNDING SOURCES

Contributors

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All other work conducted for the thesis was completed independently by me.

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NOMENCLATURE

PG	Peptidoglycan
LTG	Lytic transglycolyase

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1. INTRODUCTION

Many bacterial species move on solid surfaces. Movement of the gram-negative bacterium *Myxococcus xanthus* is facilitated by two mechanisms using independent propulsive engines: social (S-) motility and adventurous (A-) motility. S-motility, analogous to the twitching motility in *Pseudomonas aeruginosa* (Wu & Kaiser, 1995, Chang et al., 2016), is powered by the extension and retraction of type IV pili. A-motility, now defined as gliding motility, refers to bacterial locomotion on solid or semi solid surfaces unaided by flagella or pili (Nan & Zusman, 2011). The mechanism(s) of gliding have remained ambiguous for more than a century because gliding bacteria, such as *M. xanthus* and *Flavobacterium johnsoniae*, lack external structures that are connected to gliding (Spormann & Kaiser. 1999).

In *M. xanthus*, AglR, AglQ, and AglS power gliding motility using proton motive force (PMF). Among these proteins, AglR is homologous to *Escherichia coli* flagella stator protein MotA and AglQ and AglS are homologous to MotB (Nan et al., 2011, Sun et al., 2011). Unlike *E. coli* MotB that anchors on rigid peptidoglycan, AglQ and AglS lack the C-terminal peptidoglycan attachment motif. For this reason, the gliding motors, and thus the gliding complexes can move. Besides the motors formed by AglR, AglQ, and AglS, gliding also requires a series of various accessory proteins that localize in the cytoplasm, inner membrane, periplasm, and outer membrane (Nan et al., 2010, Nan et al., 2011).

Recent studies reveal that these accessory proteins usually co-localize and form complexes with the motors (Nan et al., 2010). Based on the dynamics of gliding-related proteins, a “helical rotor

model" proposes a detailed mechanism of gliding motility. In this model, the flagella stator homologs function as motors by moving in helical pathways. When the motor complexes contact the surface, they slow down, apply forces, accumulate in "focal adhesion" sites, and deform the cell surface (Nan et al., 2011). The moving distortions of the peptidoglycan (PG) layer may push cells forward against the substratum (figure 1) (Nan et al., 2011). However, it remains elusive how force generated by motor proteins transmits to the cell surface without disrupting the PG layer.

We were trying to systematically knockout all the lytic transglycosylases (LTG) for our PG project. One of the mutants, Δ*mxan_6607*, shows dramatic gliding defect. *mxan_6607* was identified by a previous screen (Youderian *et al*., 2003) as a gene involved in gliding and was named as *agmT*. But its potential LTG activity was neglected.

How does AgmT facilitate the assembly of gliding machinery? Is the LGT activity of AgmT required for gliding? To answer these questions, I aim to eliminate the LTG activity of AgmT using site-directed mutagenesis and to assay the gliding motility of the mutant strain and investigate if the mutation of AgmT affects the dynamics of AglR. At the same time, I will overexpress wildtype AgmT and determine if the excessive LTG activity will affect growth and gliding. Through these experiments, I expect to establish the connection between the gliding machinery and PG. This project will help uncover the mechanism by which *M. xanthus* transmit proton motive force to cell surface for motility.

2. LITERATURE REVIEW

Myxobacteria are Gram-negative δ-proteobacteria. In the order of Myxococcales, most species are rod-shaped soil bacteria distinguished by surface movements and fruiting body formation. *Myxococcus xanthus*, the best studied myxobacterium, is a model organism for studying surface motility, social behaviors, biofilm formation, and interspecies interaction such as predation (Zusman et al., 2007, Keane & Berleman, 2016). *M. xanthus* lacks flagella and is unable to swim in liquid. Instead, it employs two distinct mechanisms to move on surfaces: gliding and twitching (Nan & Zusman, 2011). Twitching motility in *M. xanthus* is based on the extension and retraction of type IV pili, similar to that of *Pseudomonas* and *Neisseria* (Wu & Kaiser, 1995, Chang et al., 2016). By contrast, gliding motility in *M. xanthus* appears to be unlike other characterized prokaryotic motility systems. Despite the identification of dozens of gliding-related genes (Hodgkin, 1979, Youderian et al., 2003), the search for the gliding motors lasted for decades. In 2011, two groups reported that a proton channel formed by three proteins AglR, AglQ and AglS is essential for gliding. Importantly, this proton channel/motor complex is homologous to the *Escherichia coli* flagella stator complex MotAB (AglR is a MotA homologue while AglQ and AglS are MotB homologues) as discussed above, suggesting that gliding is powered by proton motive force (PMF) (Nan et al., 2011, Sun et al., 2011). This hypothesis was confirmed by the isolation of a point mutation in the putative proton-binding site in AglQ that completely abolished gliding (Sun et al., 2011).

Since *M. xanthus* gliding does not depend on visible surface appendages, it is still an open question as to how motor proteins in the inner membrane can propagate mechanical force to the cell surface

and propel the movement of the cell body. An important clue to this enigma came from a comparison of the MotB homologues from *M. xanthus* with the *E. coli* MotB: both AglQ and AglS from *M. xanthus* lack the C-terminal peptidoglycan attachment motif. Since the *M. xanthus* AglRQS stator complex, unlike its *E. coli* homologue, was untethered, it could hypothetically be free to move within the membrane. This possibility was confirmed by direct observation of fluorescently tagged AglR using super-resolution microscopy (Nan et al., 2013). Super-resolution microscopy techniques, such as the single-particle tracking photoactivated localization microscopy (sptPALM), can pin-point the location of individual protein particles with sub-diffraction resolution (<100 nm), and to resolve real time molecular dynamics in live cells (Manley et al., 2008). To study the mechanism by which the AglRQS channel powers gliding, AglR was labeled with photoactivatable fluorophores and their molecular dynamics studied at 100-ms time resolution using sptPALM (Nan et al., 2015, Nan et al., 2013). These studies found that single AglRQS channels move in helical trajectories at up to 3-5 μm/s, indicating that rather than being restricted in the membrane, the AglRQS channel moves actively in the membrane. Collectively, the motion of hundreds of motor complexes appear as rotating helices inside each cell (Nan et al., 2015, Nan et al., 2013).

Careful analysis of the molecular behavior of the AglR protein revealed a striking phenomenon on a firm surface, the fast-moving motor complexes tend to slow down and accumulate at a few "traffic jam" sites on the ventral sides of cells, where the cells contact the gliding surface (Nan et al., 2013). These sites are dynamic as motor complexes continuously enter and leave the clusters. The clusters distribute evenly along the cell body due to helix periodicity and appear to remain near stationary as cells move forward (Nan et al., 2011, Nan et al., 2013) (Figure. 1).

These results help explain earlier data. By standard resolution microscopy, the fluorescently labeled proteins, AglR, AglQ and the motor-associated proteins AgmU and AglZ all appeared as blurry fluorescent patches or clusters that change shape and localization constantly. Despite their different cellular localization (AglR and AglQ in the membrane, AgmU in periplasm and AglZ in cytoplasm), when cells were moving on a solid surface all four proteins showed a common feature; they tended to aggregate into a few fluorescent spots that evenly distributed along the long cell axes. Surprisingly, when cells moved forward, these protein clusters did not move along with the cells but remained at fixed positions with respect to the substratum (Mignot et al., 2007, Nan et al., 2013, Nan et al., 2011, Nan et al., 2010, Sun et al., 2011). In other words, the cells appeared to move through these spots, a behavior similar to the eukaryotic motilities that depend on focal adhesions (Smilenov et al., 1999) (Figure. 1). When cells were placed in a liquid broth or in 1% methylcellulose, the labeled proteins appeared to decorate a rotating helix; however, these cells could not move by gliding as they lacked a solid surface (Nan et al., 2013, Nan et al., 2011).

Based on the above experimental observations, two models were proposed to interpret the aggregation of motor clusters and to explain the mechanism by which cells transform PMF from the inner membrane into mechanical forces on the cell surface.

2.1 The focal adhesion model

It interprets the aggregates of motor complexes as rigid focal adhesion clusters (FACs). According to this model, each locus contains multiple FACs that span the cell envelope and anchor to the substratum. The gliding motor complexes push against FACs, and thus transport these FACs linearly towards the posterior end of the cells. Since FACs and adhesins are proposed to anchor cells to the gliding surface, the backward

translocation of FACs would propel cells forward (Mignot et al., 2007, Sun et al., 2011) (Figure. 1). The exact composition of the putative FACs is still unknown. However, dozens of proteins were found to associate with the gliding complexes, including cytoplasmic, periplasmic, and integral membrane proteins and lipoproteins that attach to the inner and outer membrane (Luciano et al., 2011, Nan et al., 2010, Jakobczak et al., 2015, Youderian et al., 2003). A possible problem encountered by the focal adhesion model is the breaching of the cell wall, as the FACs are proposed to repeatedly sever the rigid peptidoglycan layer in order to push the cell body forward. However, it is possible that cells have evolved a novel mechanism to circumvent the cell wall problem, which has not yet been recognized. Over 40 genes have been reported as important for gliding motility in *M. xanthus*, but most of these genes have functions that have not yet been determined.

2.2 The helical rotor model

It proposes that the seemingly stationary fluorescence spots seen in gliding cells on surfaces are caused by the transient accumulation of motor complexes caught in dynamic "traffic jams." According to the model, the motor complexes and associated proteins move rapidly in a helical pathway through the membrane, temporarily slowing down when encountering resistance from the gliding substratum. Evidence for these "traffic jams" comes from the movement of motor complexes in cells placed on agar of different composition. On harder agar, clusters of motor complexes appear larger and individual motor complexes slow down significantly. However, upon leaving the cluster sites, their maximal velocity is restored (Nan et al., 2013, Nan et al., 2010). The accumulated motor complexes in these traffic jam sites (and their associated proteins) are proposed to exert a force that slightly deforms the cell envelope, generating a backward surface wave as the motor complexes push backward, analogous to a crawling snail. Accordingly, these traffic jam sites would act as force generators to propel the cells forward (Nan et al., 2014, Nan & Zusman,

2011). For detailed computer simulation, see (Nan et al., 2011) (Figure. 1). Indeed, regular spaced surface distortions were visualized using total internal reflection fluorescence microscopy (Nan et al., 2011) and scanning EM (Lunsdorf & Schairer, 2001, Pelling et al., 2005).

According to biophysical modeling, this mechanism should provide enough thrust to move the cells forward while avoiding breaching the cell wall (Nan et al., 2011). It is worth noting that the helical rotor model does require adhesion between the cell surface and the gliding substratum. First, adhesive materials such as slime are required to allow the helical waves to transmit the propulsive force to the substrate (Nan et al., 2011, Nan et al., 2014). Second, according to computational modeling, a certain degree of surface adhesion is required for the maintenance of gliding direction (Balagam et al., 2014).

The even distribution of the aggregates of motor proteins and the helical motion of the motor complexes both suggest the involvement of a helical structure in the cell (Mignot et al., 2007, Nan et al., 2013). In fact, MreB, the bacterial actin homologue that has the potential to form helical patches keeping the rod shape bacterial essential for gliding motility in *M. xanthus* (Mauriello et al., 2010, Nan et al., 2013, Nan et al., 2011, Treuner-Lange et al., 2015). The *M. xanthus* MreB filaments appear as fragmented filaments that display helicity when stained with antibody-conjugated fluorescent dyes (Mauriello et al., 2010). MreB filaments from *M. xanthus* are likely to differ from homologues from some other bacteria as helical MreB was not observed in *Bacillus subtilis* and *E. coli* (Dominguez-Escobar et al., 2011, Garner et al., 2011, van Teeffelen et al., 2011). Insights on MreB, such as its structure, dynamics and interaction with the gliding complex will provide critical information for understanding the mechanism of gliding. Importantly, since MreB is also a central player in cell wall synthesis (Errington, 2015), *M. xanthus* MreB must

possess unique versatility to operate on different spatial and temporal scales to orchestrate multiple functions within the same cell.

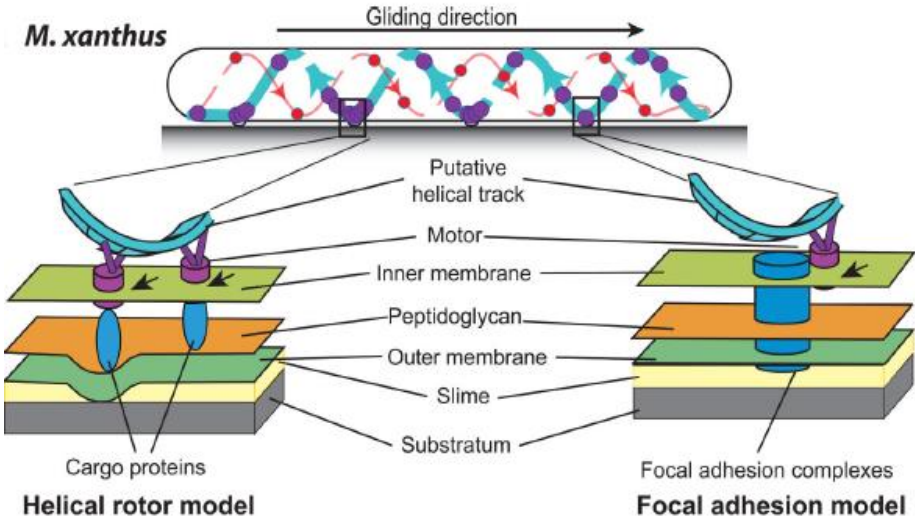

Figure 1. Models for gliding motility in *M. xanthus*, Gliding in *M. xanthus* is powered by MotAB homologues that move along helical tracks in the inner membrane. Two models propose different mechanisms by which cells transform the proton motive force from the inner membrane into a mechanical force on the cell surface. The motors push against the looped helical track (gray band) at the sites where cells envelope is in contact with the substratum. As a result, the motor complexes slow down and aggregate into protein clusters, which appear as focal adhesion sites in previous reports (Sun et al., 2011).

3. MATERIALS AND METHODS

3.1 Bacterial Strains and Gliding Assay

Strains and plasmids used in this study are listed as *DZ2*, *agmT*, *agmT*^{SM} Δ*agmT*, *agmTOE* Δ*agmT* *aglR-PAmCherry*, *aglQS*, *agmT-PAmCherry* pBJ113, pMR3629-*PAmCherry* and pMR3679-*PAmCherry*. *M. xanthus* strains were cultured in CYE medium, which contains 10 mM MOPS at pH 7.6,1% (wt/vol) Bacto Casitone (BD Biosciences), 0.5% Bacto yeast extract and 4 mM MgSO4 (29). Five-microliter 4×108 cfu·ml-1 vegetative cultures were subjected to microscope observation directly for the observation of cells suspended in liquid culture or spotted on a thin layer of 1/2 CTT agar pad containing 1.5% (wt/vol) agar for the observation of cells gliding on agar.

3.2 Construction and Analysis of *agmT* In-Frame Deletion

To make an in-frame deletion of *agmT* gene, we constructed the vector of pBJ113-*agmT* (kan^{r}), digested the left flank fragment with *Hind*III and *BamH*I and right flank fragment with *Kpn*I and *Eco*RI to construct the insert from the genome and ligate to the plasmid. I selected the colonies that underwent homologous recombination that had the original *agmT* gene deleted by colony PCR.

3.3 Fluorescence Microscopy

For regular fluorescent and photoactivatable localization microscopy (PALM) experiments, 300 μl melted agar was dropped onto a microscope slide and covered by another slide. One slide was removed after the agar solidified, leaving the agar pad on the other slide. Cells were grown in CYE

medium to OD600 ≤ 1, and 5-μl culture was dropped onto each agar pad and covered by a coverslip for single molecule imaging.

The imaging for single molecule tracking was done on an inverted Nikon Eclipse-Ti microscope with a 100× 1.49 NA TIRF objective and the images were collected using an electron-multiplied CCD camera (Hamamatsu ImagEx2, effected pixel size ~160 nm). The photoactivable mCherry was activated using a 405-nm laser and were excited and imaged using a 561-nm laser. Images were acquired at 100-ms intervals.

4. RESULTS AND DISCUSSION

4.1 The re-discovery of AgmT

The *agmT* gene was first identified to be required for gliding motility in a mutagenesis screen and its product was annotated as a "periplasmic solute-binding protein" (Wu & Kiaser,1995). However, it was discovered that the sequence of AgmT shows significant similarity with lytic transglycosylases (LTG) that are related to the integrity of PG. Importantly, the active site for LTG activity is conserved in AgmT (Figure2).

Figure 2. AgmT is predicted to be a LTG. The conserved active sites are highlighted in yellow. Source: NCB1 website

4.2 AgmT is required for gliding in *M. xanthus*

To study the function of AgmT in gliding motility, *M. xanthus* strain was constructed in which *agmT* was deleted from the chromosome. To assay the gliding motility of the Δ*agmT* strain, twitching motility was eliminated by disrupting the *pilA* gene that encodes for PilA, the pilin subunit in the pilus. The cells were grown to mid-exponential phase in rich medium, and 5ul of the culture was spotted on solid agar containing ½ CTT medium (1.5% agar, 0.5%Casitone, 10 mM Tris, 8 mM $MgSO_4$, 1 mM K_3PO_4). After a 24-h incubation, *pilA*$^-$ cells still moved outward at the edges of colonies, which indicates the gliding motility is still functional. In contrast, the Δ*agmT pilA*$^-$ cells were not able to move, indicating the absence of gliding (Figure3). Taken together, AgmT, a putative LTG, is required for gliding in *M. xanthus*.

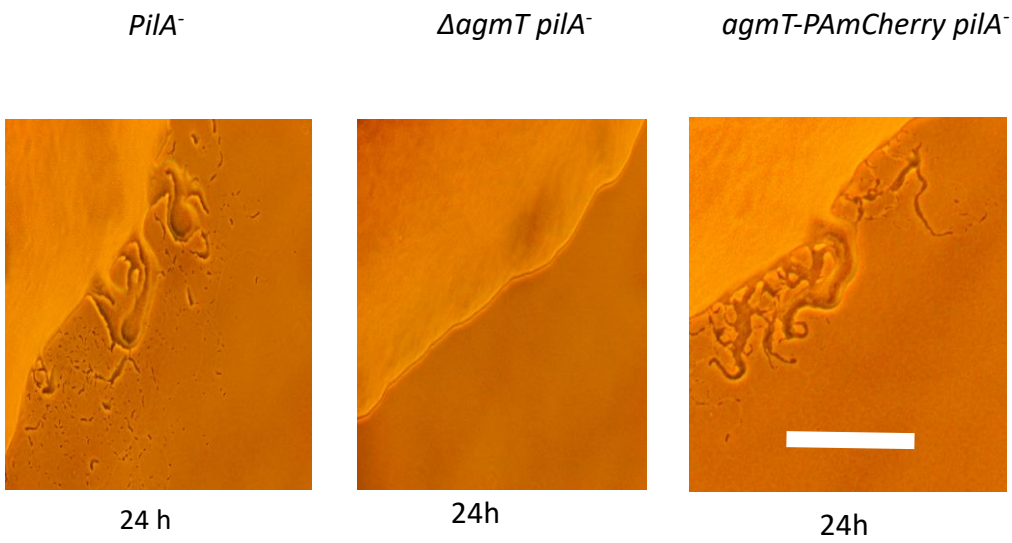

Figure 3. AgmT is required for the gliding motility of *M. xanthus*. To solely display gliding motility, the type IV pili powered twitching motility was eliminated by an insertion into the *pilA* gene, which encodes pilin, the building block of pilus. Δ*agmT pilA*$^-$ cells are not able to move away from the colony edge, indicating the lack of gliding motility. In contrast, the cells expressing photo-activatable mCherry (PAmCherry)-labeled AgmT can glide. (Scale bar, 1:100 μm).

4.3 AgmT is required for the assembly of the gliding machinery.

What is the function of AgmT in gliding? To answer this question, AgmT was labeled using photo-activatable mCherry (PAmCherry) and expressed the recombinant protein using the native locus and promoter of the *agmT* gene. The resulted strain displayed functional gliding motility, indicated that the labeled protein is functional (Figure 3 from right). Laser intensity of 405-nm excitation (0.2 kW/cm^2) was used to activate the fluorescence of a few labeled AgmT randomly in each cell and imaged single molecules at 10 Hz using single particle tracking photo-activated localization microscopy (sptPALM) (Nan et al., 2013, Nan et al., 2010). To analyze the data, fluorescent particles that remained in focus for 4 - 12 frames (0.4 - 1.2 s) were selected. Similar to AglR (Nan et al., 2010) single molecules of AgmT showed two distinct dynamic patterns. The immobile particles remained within a single pixel (160 nm ×160 nm) before photo-bleach and the mobile ones displayed either directed or diffusive motion (Figure 4). For motor proteins such as AglR, the immobile population represents the molecules that are assembled into force-generating gliding machinery in the "focal adhesion sites" whereas the mobile population contains the molecules that move between these sites (Sun et al., 2011, Nan et al., 2010). Such mobile molecules are not yet assembled into fully functional gliding machinery and thus do not contribute to gliding directly. However, compared to AglR molecules that 32% (n = 2700) were immobile, AgmT only showed 12% (n = 2989) immobile population. Importantly, the dynamics of AgmT remained unchanged in the Δ*aglQS* background where the gliding motor is truncated by the absence of both MotB homologs (Figure 5). These results indicate that AgmT does not form a stable connection with the gliding motor.

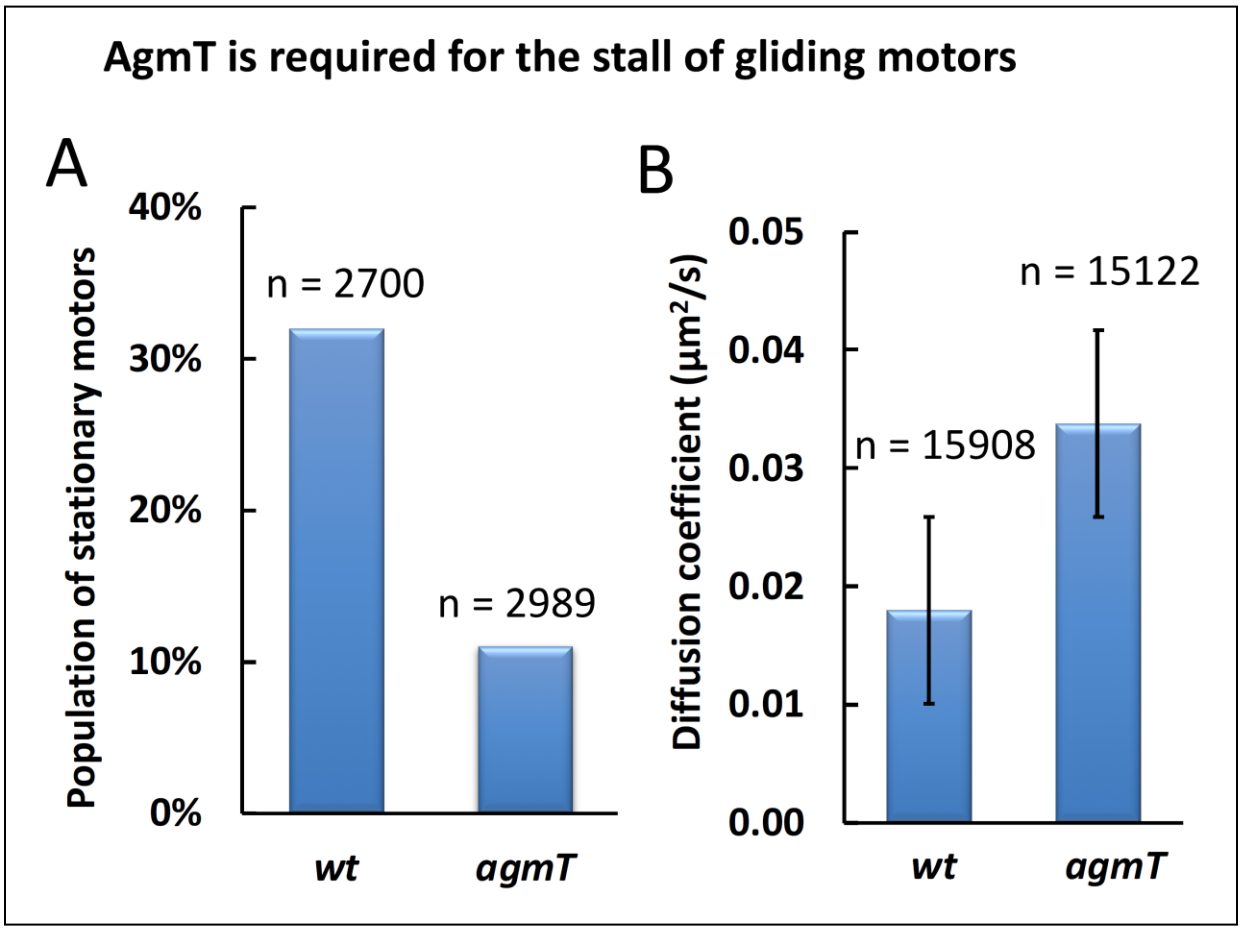

Figure 4. **AgmT is required for the assembly of force-generating gliding machinery. (A)** The motor protein AglR cannot assemble into functional (immobile) gliding machinery in the absence of AgmT. **(B)** Deletion of *agmT* increases the diffusion coefficient of mobile AglR molecules.

To test if AgmT is required for the assembly of the gliding machinery, a comparison analysis of the single-molecule dynamics of AglR in the presence and absence of AgmT was conducted. Strikingly, the absence of AgmT reduced the immobile population of AglR from 32% to 11% (n = 2989) (Figure 4). In agreement with this result, the diffusion coefficient of AglR showed a dramatic increase, from $1.8 \times 10^{-2} \pm 3.62 \times 10^{-3}$ $\mu m^2/s$ (n = 15908) to $3.4 \times 10^{-2} \pm 5.06 \times 10^{-3}$ $\mu m^2/s$ (n = 15122) (Figure 4). Taken together, these results suggest that AgmT is required for the assembly of force-generating gliding machinery.

4.4 The gliding motor does not regulate the dynamic of AgmT

The transformation of *agmT*-PAmCherry into the wild type as well as Δ*aglQS* strain was carried out to figured out the dynamic of single molecules of and dependent relationship between *agmT* and the other gliding motor. The results show *agmT* in wild type and the deletion of *aglQS* has no effect on the wild type at the stationary phase with 10% and 9.5% for the wild type and *aglQS* respectively (Figure 5 (A). In addition, Figure 5 (B) shows that the diffusion coefficient does not depend on the gliding motors with both the wild type and Δ*aglQS* with $3.4 \times 10^{-2} \pm 5.06 \times 10^{-3}$ $\mu m^2/s$. Conclusively, the dynamic of AgmT regulate AglR but AglR do not regulate AgmT.

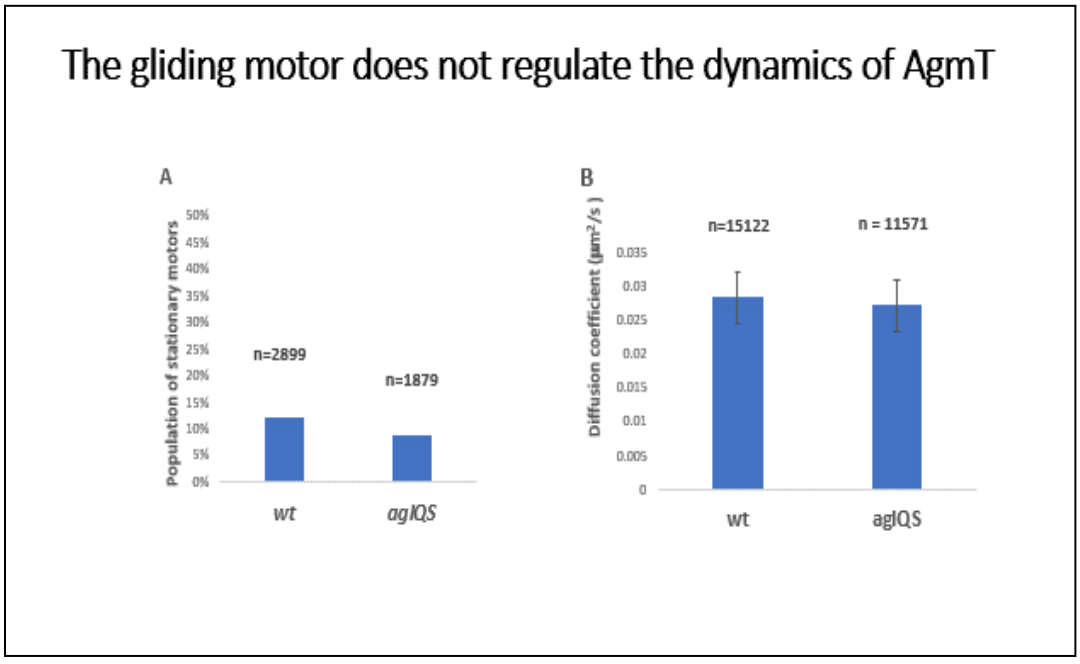

Figure 5, The gliding motor does not regulate the dynamics of AgmT. **(A)** The gliding protein Δ*aglQS* does not regulate AgmT, AgmT regulate gliding motor at the stationary phase. **(B)** The diffusion coefficient of mobile wild type does not depend on the gliding motors.

4.5 Is AgmT a LTG?

The morphogenesis analysis shows that AgmT does affect cell length. The analysis considered the length and width of the *agmT, agmTOE* and WT strains showing that t-test statistical p-value of <0.001 are statistically significant (Figure 6).

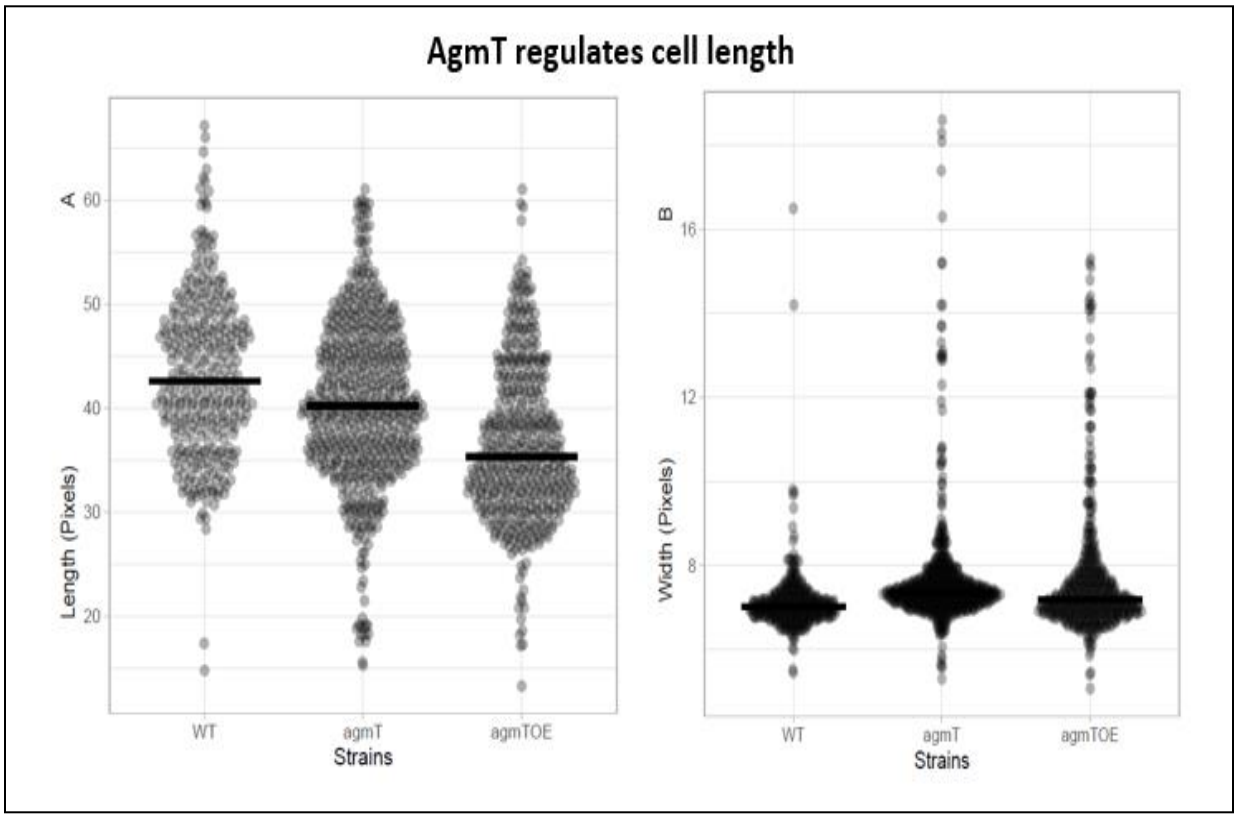

Figure 6, AgmT regulates cell length. The result of the growth curve show that AgmT does not affect cell growth as seen in panel A and B length and width respectively.

4.6 AgmT does not affect cell growth

With the aid of vanilate inducible promoter, we constructed M. xanthus strains growth analysis *agmT*, *agmTOE* and *WT* with and without vanilate (figure 7). The growth curve analysis was measured by optical density of 2-h to 24-h for the wild type and AgmT. On average value of optical density of the 3-replicates for the WT and AgmT show that both the lag phase, exponential phase and stationary phase growth look alike. This is enough to justify that AgmT is an enzyme as gliding protein. The result shows no significant difference between the two phenotypes.

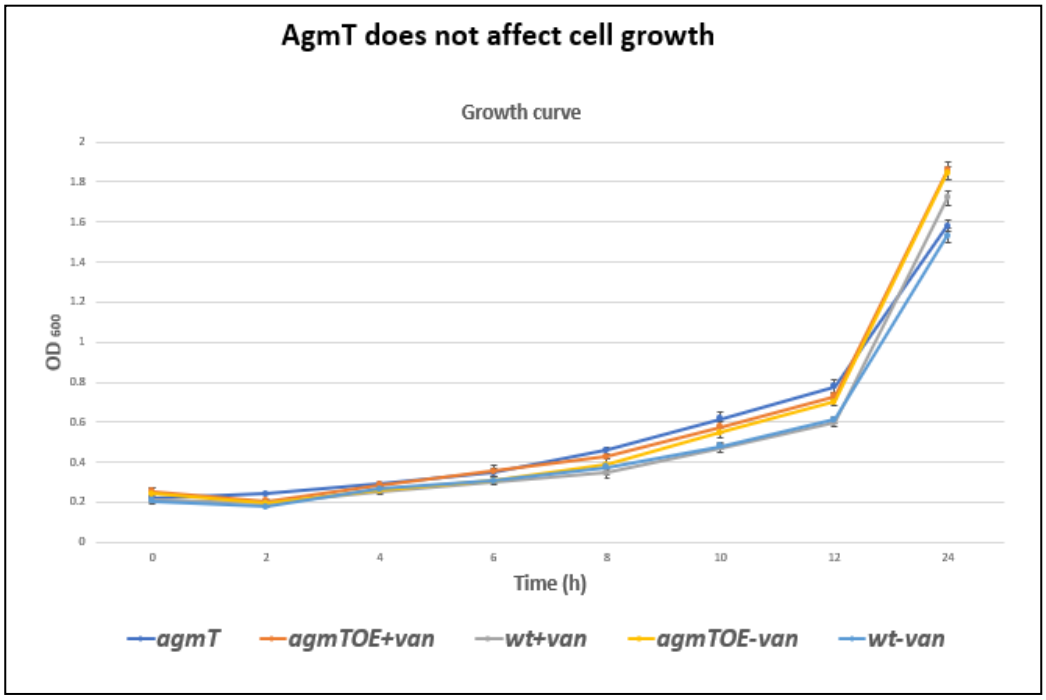

Figure 7, AgmT does not affect cell growth. Growth curve analysis of *agmT*, *agmT*^{OE} and *wt* under vanilate inducer. Shows that there is no growth arrest of *agmT*, *agmT*^{OE} with and without vanilate as compared to the empty vector (WT).

4.7 Significance of the study

The gram-negative bacterium *Myxcoccus xanthus* glides on solid surfaces utilizing an inner-membrane proton channel as the motor. Motor units, together with other accessory proteins, form static complexes that exert force between internal helical tracks and the substratum and drive a corkscrew-like motion of the tracks. As a result, the cell also moves forward like a corkscrew. After transient stalls, static complexes quickly disassemble and resume rapid motion. However, is not clear how the mechanical force transmits to cell surfaces across the rigid peptidoglycan (PG) cell wall. Here we show that AgmT, a putative lytic transglycosylase for PG, is an essential component of the gliding machinery. Using single-molecule microscopy, we found that the motors move normally in the absence of AgmT but fail to stall. Thus, we have identified the connection between the gliding motor and the PG. I propose to investigate how AgmT interacts with PG and if its transglycosylase activity is required for gliding. The findings of this project will reveal the mechanism by which *M. xanthus* transmits proton motive force from the inner-membrane to cell surface.

4.7.1 Future direction

The large periplasmic domain of AgmT seems ideal for sensing LTG as required for gliding. This region contains glutamic acid. I want to do the modification to site directed mutation experiment. Many catalytic domains of lytic transglycosylases, for example that of the soluble lytic transglycosylase Slt70 from E. coli, possess a similar fold as AgmT, which also has an acid glutamate residue in the active site (Thunnissen et al.,1995a). To test this, AgmT mutants encoding individual glutamic acid -to -alanine substitutions were expressed with a wild-type AgmT allele on the chromosome at the active site. This is observed in the blast sequence of figure 2 where LTG

are conserved at the active site. Our ongoing investigation aims to elucidate how AgmT interacts with PG and if its transglycosylase activity is required for gliding. Our findings will reveal the mechanism by which *M. xanthus* transmits proton motive force from the inner-membrane to cell surface. The data predicted will support which mechanism is supported by the model. If the mutant protein still supports gliding motility, then it's a functional protein. The helical motor is incorrect if the LG activity can physically penetrate the PG layer. We do not know. If the active site is deleted and the cell cannot move at all, that means the motor complex needs to digest the PG, at least degrade the PG partially to make it work. But, if the mutant protein still functional than means the protein is vital or associate to the PG.

5. CONCLUSIONS

AgmT is putatively required for gliding machinery, in the absence of AgmT, cell movement does not occur. AgmT does not affect cell growth, therefore this justifies that AgmT is an enzyme as gliding protein. Evidence had proved that motor units, together with other accessory proteins, form static complexes that exert force between internal helical tracks and the substratum layer and drive a corkscrew-like motion of the tracks. As a result, the cell also moves forward like a corkscrew. After transient stalls, static complexes quickly disassemble and resume rapid motion. However, is not clear how the mechanical force transmits to cell surfaces across the rigid peptidoglycan (PG) cell wall. However, the future outcome of site directed mutagenesis will determine whether the motor complex needs to digest the PG, at least degrade (deformed) the PG partially to make it work by helical rotor model or penetrate the PG from the inner-membrane to the outer-membrane by focal adhesion model. However, if the mutant protein still functional than means the protein is associated to the PG layer.

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