Structure/function analysis of the *Staphylococcus aureus* PutP protein

Matt McFarlane

Faculty Advisor: Dr. William Schwan, Ph.D., Department of Microbiology

ABSTRACT

Staphylococcus aureus causes a variety of diseases in humans and other animals. Antibiotics used to treat the infections are becoming less effective due to drug resistance. Thus, new drugs must be found, possibly targeting proline transporters. *Staphylococcus aureus* must import proline and two separate proline transporters have been recognized. The *putP* gene encodes the high affinity proline transporter used in environments low in proline. Further characterization of the *put*P protein is critical for assessing which amino acids are important for proline transport. To achieve this aim, a low stringency PCR was used to randomly mutate the *putP* gene. Truncated versions of the *putP* gene at the 3' end were also generated, giving rise to truncated PutP proteins at the carboxy terminus. The mutated and truncated *putP* PCR products were ligated onto high copy number plasmids and transformed into an *Escherichia coli* strain lacking all proline transporter genes. Three clones with a mutated *putP* gene showed diminished growth in minimal medium. The plasmids from each strain will be sequenced to determine where the mutation in *putP* has arisen. This information could be useful in determining which amino acids are essential for proper proline transport by PutP.

INTRODUCTION

Staphylococcus aureus infections, or "Staph infections", are a significant cause of human and animal disease around the world. These bacteria can cause afflictions ranging from food poisoning and skin abscesses, to bacteremias and endocarditis (6). In fact, *S. aureus* is believed to be the cause of one third of all food borne illnesses in the U.S. Of more concern, *S. aureus* has become the number one cause of hospital-acquired infections (6). Unfortunately, antibiotic resistance has also become an increasing problem, with MRSA (methicillin-resistant *S. aureus)* and VRSA (vancomycin-resistant *S. aureus*) strains being isolated (3, 9). For some VRSA, no known chemotherapeutic agent is effective. This is why new antibiotics targeting *S. aureus* need to be developed.

Gram negative enteric bacteria *Escherichia coli* and *Salmonella typhimurium* have also been shown contain a proline transporter gene similar to *putP* in *S. aureus* (8). Interestingly, these enteric bacteria have a much narrower osmotic growth range, which suggest that the proline transporter systems of *S. aureus* are able to function better at high and low osmotic environments. Since the ability of the proline transport systems in *S. aureus* may account for this ability to grow in such high osmotic conditions, an understanding of function at the molecular level would also be beneficial.

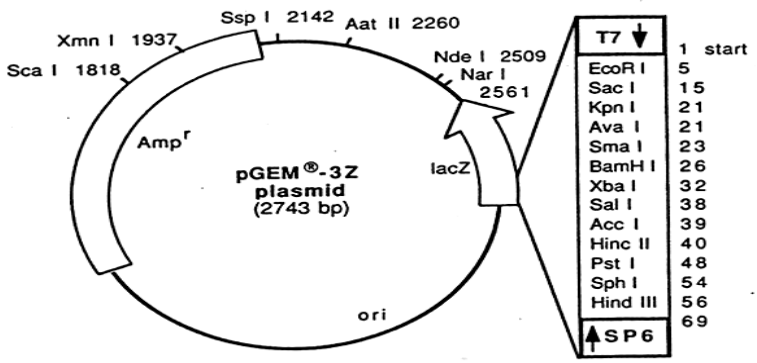
Previous studies, using animal models of infection, have demonstrated that proline transport is necessary for the survival of *S. aureus* during an infection (1, 5). *S. aureus* has two different types of proline transport systems: a low-affinity transporter and a high-affinity transporter encoded by the *put*P gene (7, 8). It has been demonstrated that bacteria possessing a mutated *put*P gene survive less well in animals, suggesting that the high-affinity proline transport system is vital for causing disease in humans (1, 3).

Understanding how the PutP proline transporter works is the first step in developing a new anti-staphylococcal drug targeting proline transport. Specific amino acids in the PutP protein need to be identified which are vital to its proper functioning. Once these specific amino acids are identified, a new anti-staphlococcal drug could be developed to target these important amino acids.

METHODS AND MATERIALS

Strains, plasmids, and Growth Conditions

S. aureus strain 6390 was used as template for the *putP* PCRs. *E. coli* strain DH5a was used to clone the full length unmutagenized *putP* gene. *E. coli* strain WG389, which has had all native proline transport genes deleted, was used to create a complemented clone with the full length *putP* gene and used to screen all truncated and low stringency *putP* PCR gene products. Transformed DH5a clones were grown on Luria agar and Luria broth containing ampicillin at 100 µg/ml for 18-24 hrs at 37°C. M9 minimal media (Janet Wood's formulation) containing ampicillin at 100 µg/ml and 25 µM or 25 mM proline was used to screen *E. coli* strain WG389 transformants grown at 37°C for 18-24 hr.



Construction of *putP* mutants

Low stringency PCR using 0.25 mM MnCl2 was used to decrease template specificity of Taq polymerase. A low stringency PCR using 0.25 mM MnCl2 and reduced dNTP concentrations were used to incorporate random errors in the *putP* gene products.

To achieve reduced dNTP concentrations, three of the nucleotides were added at 25 mM and the fourth nucleotide was added at 2.5 mM**.**

RESULTS

Table 1. Transformed WG389 colonies screened using low stringency PCR conditions.

PCR	Patched colonies [c]	Broth screened colonies [d]	Future sequencing
$MnCl_2$ [a]	700	6	0
Altered dNTP and $MnCl_2$ [b]	950	14	3

[a] $MnCl_2$ was added at a conc. of 0.25 µM to these PCRs to incorporate random errors in the *putP* gene.
[b] Three of the dNTPs in these PCRs were at normal conc. while the final dNTP was held at a lower conc. along with 0.25 µM $MnCl_2$.
[c] Colonies were patched on M9 minimal media containing 25 µM prolline and LA containing 25 mM proline.
[d] Colonies were grown in M9 minimal broth containing 25 µM or 25 mM proline.

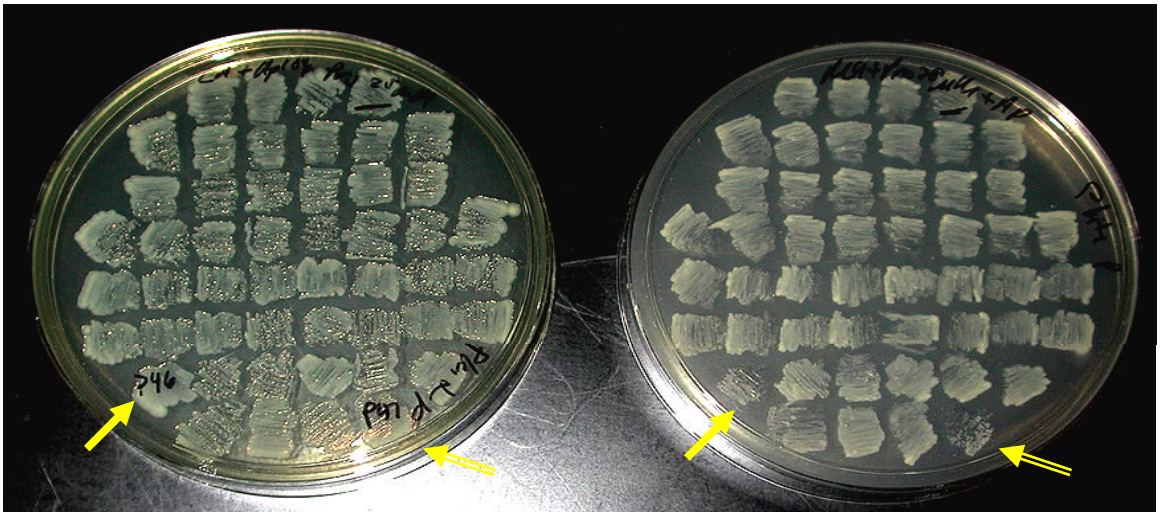


FIG. 1. Initial screen used to identify low stringency PCR *putP* mutants. Plate A is LA containing 25 mM proline. On the right, Plate B is M9 minimal media containing 25 µM proline. Clones that show inhibited growth on minimal media were moved onto a second screen in broth media.

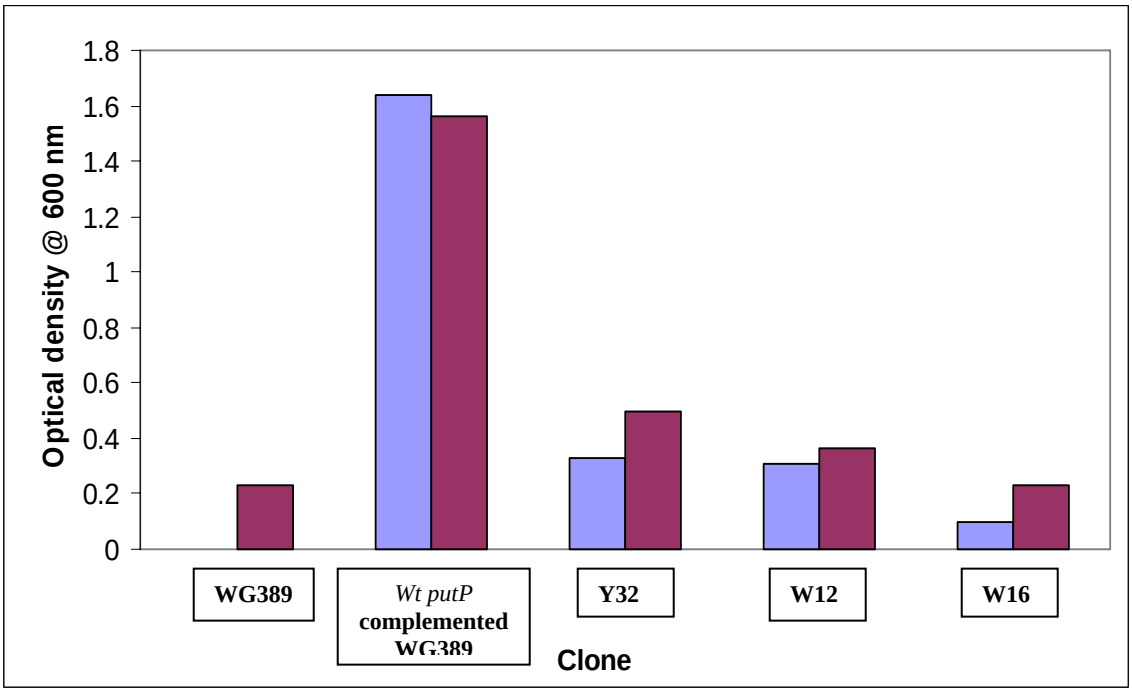


FIG. 3. Growth of *E. coli* strain WG389, wt *putP* complemented WG389, and three putative *putP* mutants in M9 minimal broth media containing 25 µM (blue) or 25 mM proline (purple). The wt *putP* complemented WG389 strain showed good growth in both low and high proline concentration media, correlating to a fully functional PutP protein. The three putative mutants displayed an inability to grow at both low proline and high proline concentrations. However, growth at the 25 mM proline concentration correlated with that of WG389 at high proline concentrations.

DISCUSSION

By using low stringency PCR, a *putP* mutant library was created and screen using *E. coli* strain WG389. Screening of over 1500 mutants lead to the isolation of three possible PutP mutants which were deficient at high affinity proline transport. After further characterization, one of these mutants, W12, had its plasmid isolated and *putP* gene sequenced to elucidate any amino acid changes which could account for the diminished proline transport phenotype. Unfortunately, the sequencing data was not sufficient enough to determine where and if any amino acid changes occurred that could account for the decreased function. Truncated *putP* PCR products at the carboxy terminus were also constructed to asses the structure and function of the PutP protein. However, attempts to clone these genes into strain WG389 failed and the necessary mutants could not be isolated. These truncations would have been useful in uncovering more data on the structure and function of the proline transporter.

Previous work on the PutP protein in *E. coli* has lead to the discovery of the important amino acids necessary for transport. Although there are significant differences in the regulation of *putP* in *E. coli* and *S. aureus,* due to the homology of the two proteins in both species, a site-directed mutagenesis approach might be an efficient and more direct approach to elucidate the important amino acids of the PutP permease in *S. aureus.* While it is expected that some differences in the proteins function exist due to the gram negative nature of *E. coli*, there is most likely conserved amino acids at the critical sites for proline transport.

Due to the increasing problem of antibiotic resistance, novel drugs and drug targets must be discovered. One possible target in *S. aureus* is proline transport, and more specifically the PutP protein. So far, the structure function analysis has not revealed any critical data to further our understanding. However, continual research of the structure and function of this protein may be vital for designing or finding a novel anti-staphlococcal drug.

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