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MINIREVIEWS

Osmolyte transport in *Staphylococcus aureus* and the role in pathogenesis

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Abstract

Osmolyte transport is a pivotal part of bacterial life, particularly in high salt environments. Several low and high affinity osmolyte transport systems have been identified in various bacterial species. A lot of research has centered on characterizing the osmolyte transport systems of Gram-negative bacteria, but less has been done to characterize the same transport systems in Gram-positive bacteria. This review will focus on the previous work that has been done to understand the osmolyte transport systems in the species *Staphylococcus aureus* and how these transporters may serve dual functions in allowing the bacteria to survive and grow in a variety of environments, including on the surface or within humans or other animals.

Key words: *PutP*; *OpuD*; *Staphylococcus aureus*; Proline transport; Osmolyte

Core tip: *Staphylococcus aureus* (*S. aureus*) is the number one cause of skin and soft tissue infections. In the United States, *S. aureus* is usually the number one hospital-acquired pathogen. The skin and urinary tract organs are high osmotic stress environments. Osmolyte transport is essential for *S. aureus* survival in different environmental niches, such as within human skin abscesses or the human urinary tract.

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INTRODUCTION

A well conserved, evolutionary strategy used by many organisms to adapt to high osmotic conditions is the transport of organic compounds, called compatible solutes[1]. These compatible solutes serve as cytoplasmic solutes that balance water relations, without interfering with normal cytoplasmic activities, within cells grown in high salt environments. Examination of the transport

systems in *Staphylococcus aureus (S. aureus)* may provide insight into how proline and glycine betaine may be transported into Gram-positive bacteria.

GENERAL OSMOLYTE TRANSPORT FEATURES IN *S. AUREUS*

Although osmolyte transport is best described in *E. coli*[1-3], there are also compatible solute transport systems in *S. aureus* to adapt to high salt environments[4]. Studies have shown that *S. aureus* cells grown in very high salt environments had increased intracellular levels of proline and glycine betaine[5-11]. Other intracellular molecules that also increased in high NaCl environments were choline, proline betaine, taurine, and glutamic acid[6,7,12]. Of these accumulated solutes, proline and glycine betaine were the most effective osmoprotectants of *S. aureus*, since *S. aureus* growth was observed when these solutes were excluded from defined high osmotic media[6,8,12].

Identification of genes that encode transport proteins and their importance for the survival of *S. aureus* coincides with previous observations that *S. aureus* requires several amino acids as a source of carbon and nitrogen[4]. Of these essential amino acids, proline and other amino acids are not synthesized by *S. aureus*[4,13,14]. The accumulation of most of the proline in *S. aureus* occurs because of proline transport proteins.

Although prior research performed using other Gram-positive bacteria may not have specifically addressed proline transport, it does help in uncovering commonly conserved mechanisms of compatible solute transport in *S. aureus*. Several studies that have examined compatible solutes accumulation in *S. aureus* grown at high osmotic environments showed increased intracellular levels of proline, aminobutyric acid, glutamic acid, choline, taurine, and glycine betaine[5-7,15,16]. Of these compatible solutes, only glutamic acid is synthesized by *S. aureus*, whereas the other compatible solutes have to be imported from the external environment[5,7,8,17-19]. To substantiate the osmoprotective importance of these transported compatible solutes, the growth rates of *S. aureus* grown in defined high osmotic media was observed to increase when supplemented with either proline or glycine betaine[8]. Although *S. aureus* normally possess relatively large concentrations of glycine betaine and potassium ions, compatible solute transport is believed to aid in creating high intracellular pressure that enables *S. aureus* to survive in high osmotic environments[15].

SPECIFIC PROLINE TRANSPORT SYSTEMS IN *S. AUREUS*

Initial proline uptake research using whole cell assays on *S. aureus* has shown the presence of at least two proline transport systems[10,17,20]: Both a low- and high-affinity system. These systems may be similar to the OpuE and OpuD transport systems found in *B. subtilis*[21,22] and they share properties with the PutP and ProP systems of *E. coli*[1]. They are both sodium-dependent transporters, since gramicidin D and monensin, which collapse Na^+ gradients, inhibit proline transport in both systems[10]. Proline transport in either system showed low susceptibility to inhibition by glycolysis and ATP formation by a combination of NaF and sodium iodoacetate or sodium arsenate, respectively. Lastly, alterations of pH from 5.5 to 8.5 had little effect on the transport rates of proline[10].

In *S. aureus*, proline transport kinetics is hard to interpret because of strain differences and the calculation setups used to determine the K_m and V_{max} values reported, one based on per mg protein and the other per mg dry weight. Reports have shown that the high-affinity proline transport system in *S. aureus* had a K_m ranging from 1.7 to 7.0 mol/L, with a V_{max} ranging from 1.1 nmol/min per milligram dry weight to 10 nmol/min per milligram protein[10,17]. Though these numbers are not directly comparative, they do give us a relative range of activity for this system, which correlates to a previously observed K_m value of 3.5 mol/L for proline uptake with vesicles prepared from *S. aureus* grown in a low-osmolarity medium[23] and K_m values of the PutP system in *E. coli*[1,17,24-26]. Moreover, like the PutP system of *E. coli*[1], the high-affinity proline transport system in *S. aureus* is specific for the transport of proline and it's activity increases when proline deprivation is encountered, suggesting that this system may also be involved in scavenging low concentrations of proline from the environment[10]. Further proof of the relatedness of these systems can be seen from the complementation of a genetic defect in proline transport within *E. coli* by the high-affinity proline transport system of *S. aureus*[27]. At the structural level, the PutP homolog of *S. aureus* shows a sodium-binding motif, the same ten conserved amino acids found in all other members of the sodium/solute symporters[28], and the predicted PutP protein of *S. aureus*[29] shares considerable similarity with the PutP protein of *E. coli*[1]. Although many similarities exist between the high-affinity proline transport systems in *S. aureus* and *E. coli*, major differences between these systems include: The concentration of NaCl appears to have no effect on proline transport in *S. aureus*[8,17]; the *S. aureus putP* gene is activated by high concentrations of osmolytes in the environment[30], whereas the *E. coli putP* gene is not[1,25,29]; and the *S. aureus putP* gene is regulated by SigB[30], which is similar to the regulation shown for *opuE* in *B. subtilis*[21]. Although PutP has a sodium binding motif and has homology with sodium/solute symporters, the concentration of NaCl does not affect proline transport[7,17], It is possible that when *S. aureus* is grown in an environment with a low sodium concentration that PutP behaves like other bacterial high affinity proline transporters that are driven by a sodium motive force. On the other hand, *S. aureus* grown in a high sodium environment may cause the PutP protein to use a proton motive force instead of a sodium motive

Table 1 Distribution of proline and glycine betaine transport genes in some sequenced

	S. aureus strains			
Gene	N315	MW2	COL	Mu50
putP	SA1718	MW1843	SACOL1963	SAV1902
putP	SA0531	MW0528	SACOL0620	SAV0573
opuD	SA1183	MW1236	Yes (2)[2]	SAV1349[4]
opuD1	-[1]	-[1]	SACOL1384	ND[3]
opuD2	-[1]	-[1]	SACOL2176	ND[3]
opuCA	SA2237	MW2372	ND[3]	SAV2448
opuCB	SA2236	MW2371	ND[3]	SAV2447
opuCC	SA2235	MW2370	ND[3]	SAV2446
opuCD	SA2234	MW2369	ND[3]	SAV2445

[1]Does not possess; [2]Multiple *opuD* genes in this species; [3]Not determined; [4]The gene appears to be fragmented into two pieces.

force to bring proline into the cell.

The low-affinity proline transport system of *S. aureus* also has similarities to the low-affinity proline transport system (ProP) of *E. coli*. For proline transport, the K_m value of *S. aureus* ATCC 12600 (K_m of 420 mol/L and V_{max} of 110 nmol/min per milligram protein) is similar to the K_m value of ProP in *E. coli* (approximately 300 mol/L)[17]. For *S. aureus* (K_m of 132 mol/L and V_{max} of 22 nmol/min per milligram dry weight), a greater difference in the K_m values for the low-affinity proline transport system can be seen between strains as compared to the difference in K_m values for the high-affinity system. Again, the K_m and V_{max} values from the ProP system of *E. coli* fit within the overall range found for *S. aureus*[1,31-33], but strain variation along with calculation setup differences may again be the cause of these divergent numbers. Excluding the differences of the K_m and V_{max} values between strains, the low-affinity proline transport systems of different *S. aureus* strains possess identical characteristics[10,17]. Many of these characteristics are similar to the regulatory and functional properties of the ProP system of *E. coli*[34] (*i.e.,* both of these systems transport proline and are stimulated by increasing osmolarity produced by either ionic or nonionic solutes)[17].

DIFFERENCES IN THE *S. AUREUS* OSMOLYTE TRANSPORT SYSTEMS COMPARED TO OTHER BACTERIA

Though these systems are similar, there are some major differences between the Gram-negative and Gram-positive low-affinity proline transport systems. One major difference is that the low-affinity proline transport systems in *S. aureus* are optimally activated at NaCl concentrations ranging from 0.75 to 1.0 mol/L[17,35], whereas the low-affinity proline transport systems in *E. coli* are inhibited by NaCl concentrations greater than 0.2 to 0.3 mol/L[29,36]. Other major differences include glycine betaine transport activity by the low-affinity proline transport system has not been conclusively established and there conflicting opinions and data presented for the glycine betaine transport activity for the low-affinity system[9,17,18,20,37]. In part, the previous lack of any low-affinity system mutants in those studies complicated the examination of glycine betaine transport activities. Since glycine betaine accumulation has been linked to proline transporters in Gram-negative bacteria[1] and *S. aureus* has been shown to transport glycine betaine from the external environment[38], this suggests that an additional glycine betaine transporter that is osmotically stimulated may be present in *S. aureus*. Moreover, *S. aureus* cells shocked with 0.5 mol/L NaCl in the presence and absence of chloramphenicol (100 g/mL) showed identical levels of transported proline, suggesting that new protein synthesis is not necessary for rapid proline uptake and that osmotic shock activates a pre-existing proline transport system[10].

BIOINFORMATIC TOOLS TO IDENTIFY OSMOLYTE TRANSPORT SYSTEMS IN *S. AUREUS*

Sequencing of several *S. aureus* genomes has provided a wealth of information on the existence of several putative osmolyte transport systems in *S. aureus*[14,39,40]. All of the strains appear to have a conserved *putP* gene for high affinity transport of proline, although there appears to be homologs for both a *proP* gene[1] and *opuD* gene[21,35] (Table 1). Additional analyses have shown that the *opuD* gene (encoding a low affinity proline transporter) is activated under osmotic stress conditions and OpuD transports proline under low affinity growth conditions[35]. Furthermore, a mutation in the *S. aureus proP* gene also causes lower proline transport in media with high concentrations of proline (Schwan WR unpublished data).

This is the first instance of both the ProP and OpuD low affinity proline/glycine betaine transport homologs being identified in one species and suggests the importance that proline transport must have in the survival of *S. aureus* cells in a variety of environments. Furthermore, the *opuC* system, which putatively transports glycine betaine/carnitine/choline, has also been observed. Together, the bioinformatic comparisons have uncovered some very interesting genomic features in *S. aureus* centered on osmolyte transport. A summary of the four osmolyte transport systems in *S. aureus* tied to proline transport and other known solutes is noted in Figure 1.

OSMOLYTE TRANSPORT TIED TO *S. AUREUS* SURVIVAL IN HUMANS AND MICE

The rationale of investigating proline and glycine betaine transport in *S. aureus* is not purely academic. In planktonic *S. aureus*, the glycine betaine level is high,

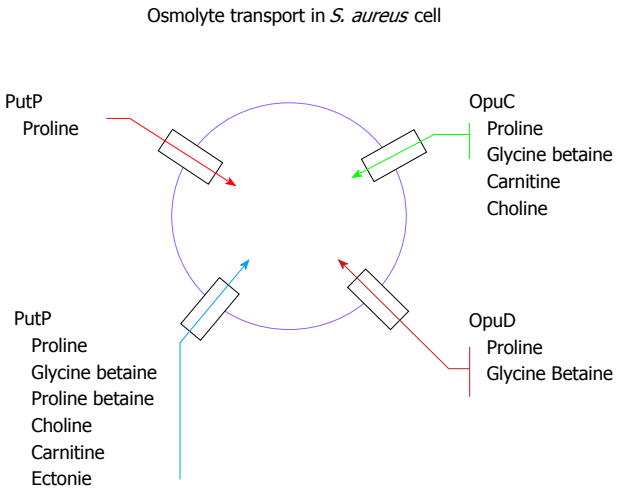

Figure 1 The four prominent osmolyte transport systems in *Staphylococcus aureus* tied to proline transport as well as other solutes.

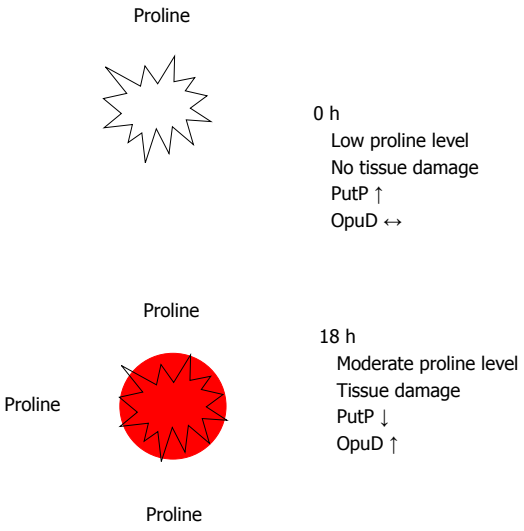

Figure 2 Model for the roles of proline transporters in *Staphylococcus aureus* pathogenesis within a murine abscess.

but lower in *S. aureus* found in biofilms[41]. Glycine betaine is the most effective osmoprotectant. To achieve the high glycine betaine level, an active glycine betaine transporter would need to be functioning in the planktonic *S. aureus* cells that are immersed in an environment of high osmotic stress, like the human skin.

Indirect effects on *S. aureus* survival have been tied to osmolyte transport systems. Defects in the cell wall caused by a *femAB* mutation caused an upregulation of *opuC* (glycine betaine/carnitine/choline transporter) and downregulation of *opuD* to compensate for the defect[42]. YhcSR encodes a two-component signal transduction system that is required for *S. aureus* survival. This two-component regulatory system regulates transcription of the *opuCABCD* operons affecting proline and glycine betaine levels in *S. aureus*[43]. One study examining daptomycin resistance revealed an accumulation of glycine betaine within *S. aureus* cells that was coupled with upregulation of the *cudT* (choline transporter) gene, a beta choline dehydrogenase gene, a *gbsA* gene (glycine betaine aldehyde dehydrogenase), an *opuD2* gene, and the *proP* gene[44]. Uptake of choline is needed to produce glycine betaine internally, the best osmoprotectant[19].

More directly, a transposon mutation in the gene for the high affinity (PutP) proline transport system of *S. aureus* rendered the bacteria less able to survive in several animal infection models[45-47]. Within cardiac vegetations, the viable *S. aureus* count was 1-3 logs lower than the wild-type parent strain[45]. Transcription of *putP* was shown to increase 105-fold shortly after *S. aureus* infection of murine kidneys[30]. In *S. aureus* infected murine bladders, spleen and livers, *putP* transcription was also elevated very quickly and then dropped markedly as the infection progressed. Proline levels in livers and spleens are very low[47] and the levels are likely low in the other organs (*e.g.*, bladder and kidney), but through tissue damage by staphylococcal toxins, the concentration of proline may increase substantially and in turn shut off transcription of the high affinity proline transport gene.

Conversely, transcription of the low affinity proline transport gene *opuD* was shown to be the highest after 4 h post-infection in murine bladders and 18 h post-infection in murine thigh abscesses[35]. Within murine bladders and kidneys, high osmotic conditions prevail. Initial observations demonstrated that at least one of the low-affinity proline transport systems of *S. aureus* was activated under moderate to high osmotic conditions[17], which has been subsequently confirmed[35].

Our model is that PutP is important in the early stages of an infection when proline concentrations are low, but OpuD expression is not as important (Figure 2). As the infection proceeds, tissue damage occurs, which releases free proline. By 18 h post-infection, the level of free proline is higher and OpuD becomes important at this stage of the infection.

These studies suggest that osmolyte transport systems may play essential roles in survival of *S. aureus* within humans or mice. Characterization of the proline and glycine betaine transport systems will provide us with experimental proof of the importance of these systems during growth in high osmotic conditions, how these systems are regulated, and will further our understanding of the significance of the proline/glycine betaine transport to the survival of *S. aureus in vivo*.

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