rho Is Not Essential for Viability or Virulence in *Staphylococcus aureus*

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We have identified the gene for transcription termination factor Rho in *Staphylococcus aureus*. Deletion of *rho* in *S. aureus* reveals that it is not essential for viability or virulence. We also searched the available bacterial genomic sequences for homologs of Rho and found that it is broadly distributed and highly conserved. Exceptions include *Streptococcus pneumoniae, Streptococcus pyogenes, Mycoplasma genitalium, Mycoplasma pneumoniae, Ureaplasma urealyticum,* and *Synechocystis* sp. strain PCC6803, all of which appear not to possess a Rho homolog. Complementation studies indicate that *S. aureus* Rho possesses the same activity as *Escherichia coli* Rho and that the Rho inhibitor bicyclomycin is active against *S. aureus* Rho. Our results explain the lack of activity of bicyclomycin against many gram-positive bacteria and raise the possibility that the essentiality of *rho* may be the exception rather than the rule.

The *rho* gene codes for transcription termination factor Rho (19) and is essential for the viability of *Escherichia coli* (5). The function of Rho is to catalyze the release of RNA from a transcription complex after a Rho-dependent terminator sequence has been transcribed. Rho has an RNA-dependent NTPase activity, which is required for transcript release (10, 12), and an RNA-DNA helicase activity (1). Several Rho-dependent transcription terminators in *Escherichia coli* and coliphage λ are known (4, 20).

Rho is a hexamer of identical 47-kDa monomers (2, 9, 11, 17). Its ability to bind RNA is thought to be conferred by residues 22 to 116 of the 419-amino-acid protein (14). The ATP binding domain is located between residues 167 and 319 (7). No activity has been assigned to the essential C terminus of Rho, although it has been speculated as being involved in subunit interactions (1, 6, 8).

The antibiotic bicyclomycin inhibits Rho (23). With the exception of *Micrococcus luteus* (15, 16), gram-positive bacteria are resistant to bicyclomycin, and *rho* is not essential in the gram-positive bacterium *Bacillus subtilis* (13, 21). This study reports the sequence and characterization of the *rho* gene in the clinically important gram-positive pathogen *Staphylococcus aureus*.

MATERIALS AND METHODS

Bacteria and growth conditions. *Staphylococcus aureus* was propagated in either tryptic soy broth (DIFCO) or Luria broth (LB). *E. coli* was grown in LB. For auxotrophy measurements with *S. aureus*, M9 medium was supplemented with arginine, cysteine, glutamate, glycine, isoleucine, leucine, methionine, proline, and valine, all at 20 μg/ml. Nicotinate and thiamine were added at 0.2 μg/ml. All cultures were routinely incubated at 37°C. All strains used in this study are described in Table 1.

Identification of the *S. aureus* rho gene. The *rho* gene from *Staphylococcus aureus* was identified from an *S. aureus* genomic sequence database by homology with *E. coli* rho. The plasmid pAU2442 used in this study is from a genomic library used in the sequencing project.

Deletion mutagenesis. A *rho* deletion was introduced into *S. aureus* RN4220. This was achieved by electroporating *S. aureus* RN4220 to tetracycline resistance (Tc^r) with pRW101, which has the *rho* gene with 500 bp of flanking sequence interrupted by a tetracyline resistance marker that replaces the *rho* gene from codon 1 to codon 313 (Fig. 1). The resulting Tc^r colonies were then screened for erythromycin resistance (Em^r), which is carried elsewhere on pRW101. Tc^r Em^s colonies indicated a double-crossover event occurred. The deletion of *rho* was confirmed by PCR analysis with primers flanking the Tc^r marker. To construct a Δ*rho* strain in a pathogenic background, the Δ*rho*::*tet* mutation was moved into the *S. aureus* clinical isolate WCUH29 by bacteriophage Φ*11* transduction. The resulting strain, RSW101, was used in virulence testing as described below.

Complemention of *E. coli* rho. *E. coli* rho was replaced with *S. aureus* rho by introducing the *S. aureus* rho-containing plasmid pAU2442 into the *E. coli* rho deletion strain BLS107(pPM*rho*). This strain has a deletion-insertion (*rho*::kan) mutation in the chromosomal copy of *rho*. A wild-type copy of *rho* is carried on the plasmid pPM*rho,* which has a temperature-sensitive replicon. This strain fails to grow at 42°C due to the loss of *rho* at this temperature (22). pAU2442 was introduced into BLS107(pPM*rho*) by electroporation and was plated at 30, 37, and 42°C. As a control, the parent strain was also plated at the different temperatures. While the parent strain failed to form isolated colonies at 37 and 42°C, the strain containing pAU2442 grew well and formed isolated colonies at all temperatures. The loss of pPM*rho* was confirmed by scoring for loss of chloramphenicol resistance, which is encoded by this plasmid. To determine if the *S. aureus* Rho protein is sensitive to bicyclomycin, strain BLS107(pAU2442) was tested for sensitivity by MIC determination. Liquid MICs were determined by a serial dilution method. Strain BLS107 was found to have an MIC of bicyclomycin

TABLE 1. Strains and plasmids used in this study

Strain or plasmid	Description	Source or reference
Strains		
BLS107	*E. coli* Δ*rho*::Kan^r	22
MG1655	Wild-type *E. coli* strain	Laboratory collection
RN4220	*S. aureus* laboratory strain	Laboratory collection
RSW100	RN4220 Δ*rho*::Tet^r	This study
RSW101	WCUH29 Δ*rho*::Tet^r	This study
WCUH29	*S. aureus* clinical isolate	Laboratory collection
Plasmids		
pAU2442	4.3-kb genomic clone from WCUH29 containing *rho*	This study
pPM*rho*	Temperature-sensitive replicon, 3.0-kb genomic clone of *E. coli* containing *rho*	22

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   1 GTCAGTTATCACTTATAACGATTTAGATGAAGCGATTCAAATTGCAAATGATACAAAATATGGTTTGGCAGGATA
  76 TGTTATTGGTAAGGACAAAGAAACATTGCATAAAGTAGCTCGTTCTATTGAAGCAGGTACAGTAGAAATAAACGA
 151 AGCAGGTAGAAAGCCAGATTTACCATTTGGTGGCTATAAACAATCTGGTTTAGGTCGTGAATGGGGCGATTATGG
 226 TATTGAAGAGTTCTTAGAAGTGAAATCTATAGCTGGATATTTTAAATAAATTTGATTTTTGAATTAAAAATCGCA
 301 ATAAAACAGTGCACATGACTAATTAAGTTTTGTGTACTGTTTTAATTTTGCAATTTTTATAAATAGATTTTGTAA
 376 TTAAAATAAAAATTTGCTATAGTTATTCATGTATTTAAAAGGTTGGGGATTAGCATAATGGGATTGTGCTAGCAC
 451 AGTTATTTATGCATTGTCATGCCTATCTATTACTTACTAACTAAAAAATAATGAAATGGGTGTAAACTATATGCC
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                                                                    M  G  V  N  Y  M  P
 526 TGAAAGAGAACGTACATCTCCTCAGTATGAATCATTCCACGAATTGTACAAGAACTATACTACCAAGGAACTCAC
       E  R  E  R  T  S  P  Q  Y  E  S  F  H  E  L  Y  K  N  Y  T  T  K  E  L  T
 601 TCAAAAAGCTAAAACTCTTAAGTTGACGAACTATAGTAAATTAAATAAAAAGAACTTGTTCTAGCTATTATGGA
       Q  K  A  K  T  L  K  L  T  N  Y  S  K  L  N  K  K  E  L  V  L  A  I  M  E
 676 AGCACAAATGGAAAAGATGGTAACTATTATATGGAAGGTATCTTAGATGATATACAACCAGATGGTTATGGTTT
       A  Q  M  E  K  D  G  N  Y  Y  M  E  G  I  L  D  D  I  Q  P  D  G  Y  G  F
 751 TTTAAGAACAGTGAACTATTCTAAAGGGGAAAAAGATATTTATATATCTGCTAGCCAAATTCGTCGTTTTGAAAT
       L  R  T  V  N  Y  S  K  G  E  K  D  I  Y  I  S  A  S  Q  I  R  R  F  E  I
 826 TAAACGTGGGGATAAAGTAACTGGGAAAGTTAGAAAACCTAAAGATAACGAAAAATATTATGGCTTATTACAAGT
       K  R  G  D  K  V  T  G  K  V  R  K  P  K  D  N  E  K  Y  Y  G  L  L  Q  V
 901 TGACTTTGTCAATGACCATAACGCAGAAGAAGTGAAGAAACGTCCGCATTTCCAAGCTTTGACACCACTTTATCC
       D  F  V  N  D  H  N  A  E  E  V  K  K  R  P  H  F  Q  A  L  T  P  L  Y  P
 976 AGATGAGCGTATTAAATTAGAGACAGAAACACAAATTATTCAACGCGCATCATGGATTTAGTAACACCGATTGG
       D  E  R  I  K  L  E  T  E  T  Q  N  Y  S  T  R  I  M  D  L  V  T  P  I  G
1051 TTTAGGTCAACGTGGTTTAATAGTGGCGCCACCTAAAGCAGGTAAAACATCGTTATTAAAAGAAATAGCGAATGC
       L  G  Q  R  G  L  I  V  A  P  P  K  A  G  K  T  S  L  L  K  E  I  A  N  A
1126 AATCAGTACGAACAAACCAGATGCAAAGCTATTTATTTTGTTAGTTGGCGAGCGTCCTGAAGAGGTGACAGATTT
       I  S  T  N  K  P  D  A  K  L  F  I  L  L  V  G  E  R  P  E  E  V  T  D  L
1201 AGAACGCTCAGTAGAAGCTGCTGAAGTCGTTCATTCAACGTTTGACGAACCACCAGAACACCATGTTAAAGTAGC
       E  R  S  V  E  A  A  E  V  V  H  S  T  F  D  E  P  P  E  H  H  V  K  V  A
1276 TGAATTATTACTTGAACGTGCAAAGCGTTTAGTAGAAATTGGGGAAGATGTCATTATTTTAATGGATTCTATAAC
       E  L  L  L  E  R  A  K  R  L  V  E  I  G  E  D  V  I  I  L  M  D  S  I  T
1351 GAGATTAGCACGCGCTTATAACTTAGTTATTCCACCAAGTGGTCGTACATTATCAGGTGGTTTAGATCCTTGCAT
       R  L  A  R  A  Y  N  L  V  I  P  P  S  G  R  T  L  S  G  G  L  D  P  C  I
1426 CTTACACAAACCAAAAGCATTCTTCGGTGCAGCGAGAAATATTGAAGCGGGTGGAAGTTTAACAATACTTGCAAC
       L  H  K  P  K  A  F  F  G  A  A  R  N  I  E  A  G  G  S  L  T  I  L  A  T
1501 TGCATTAGTTGATACGGGTTCACGTATGGACGATATGATTTACGAAGAATTTAAAGGAACAGGTAACATGGAGTT
       A  L  V  D  T  G  S  R  M  D  D  M  I  Y  E  E  F  K  G  T  G  N  M  E  L
1576 ACATTTAGATCGTAAATTGTCTGAACGTCGTATCTTCCCTGCAATTGATATTGGCAGAAGTTCAACGCGTAAAGA
       H  L  D  R  K  L  S  E  R  R  I  F  P  A  I  D  I  G  R  S  S  T  R  K  E
1651 AGAATTGTTAATAAGTAAATCTGAATTAGACACATTATGGCAATTAAGAAATCTATTCACTGACTCAACTGACTT
       E  L  L  I  S  K  S  E  L  D  T  L  W  Q  L  R  N  L  F  T  D  S  T  D  F
1726 TACTGAAAGATTTATTCGCAAACTTAAAAGGTCTAAGAATAATGAAGATTTCTTCAAGCAGCTACAAAAGTCTGC
       T  E  R  F  I  R  K  L  K  R  S  K  N  N  E  D  F  F  K  Q  L  Q  K  S  A
1801 AGAAGAAAGTACTAAAACGGGTCGACCTATAAATTTAATAAACATTATATAGGGGCTTGCGTTTTGAATTAATTAC
       E  E  S  T  K  T  G  R  P  I  I
1876 CTTTATAATTACACAGTATTGGGTAAAAACTCACAAATAACTCTGTTCCAGATGGTTCAGGGCAAAGGAGCTGAA
1951 AATAATGAAACAAG
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FIG. 1. Sequence of *S. aureus rho* and flanking sequences used in this report. Shown is the DNA sequence of the region of the *S. aureus* chromosome that contains *rho*. The *rho* gene product is shown below its coding sequence. DNA sequence with a strikethrough line depicts the sequence deleted in the *rho* deletion-insertion strain described in this paper.

of 125 μg/ml. This is twofold lower than that of the wild-type *E. coli* strain MG1655 (250 μg/ml). The MIC for the *S. aureus* strain RN4220 was found to be >2.5 mg/ml.

Virulence testing. The *rho* deletion strain RSW101 and its wild-type parent, WCUH29, were tested for virulence with two mouse *S. aureus* infection models. The surgical wound model measures the ability to grow on sutures sewn in the skin of healthy mice, while the hematogenous pyelonephritis model measures the ability of the strains to cause kidney infections following parenteral infection with the bacteria.

Surgical wound infections. The mice used for these infections were 4- to 6-week-old male CD-1 mice. Approximately 15-cm sutures were soaked in overnight cultures of WCUH29 and RSW101 for 30 min at room temperature. The backs of the mice were shaved, the animals were anesthetized with isoflurane, and an ~2-cm-long incision was made along the animals' backs. The infected sutures were secured at one end of the wound and tacked once midway along the wound on the underside of the skin. The suture was then secured at the other end of the wound, and the wound was closed with a single staple. After 5 days, the mice were sacrificed by CO_2 overdose. The skin surrounding the wound was excised and then homogenized in 1 ml of phosphate-buffered saline (PBS) in a stomacher, and bacterial viable counts were enumerated. Both infections yielded over 10^6 bacteria after 5 days, with no significant difference observed between the strains. For the hematogenous pyelonephritis model, bacterial recovery rates (mean ± standard deviation; $n = 5$) were $5.35 ± 0.41 \log_{10}$ CFU/ml for strain WCUH29 and $6.44 ± 0.81 \log_{10}$ CFU/ml for strain WCUH29 Δ*rho*. For the surgical wound model, the recovery rates were $6.58 ± 0.78 \log_{10}$ CFU/ml for strain WCUH29 and $7.41 ± 0.57 \log_{10}$ CFU/ml for strain WCUH29 Δ*rho*. One

S. aureus Rho   MGVNYMPERERTSPQYESFHELYKNYTTKELTQKAKTLKLTNYSKLNKKELVLAIMEAQM
B. subtilis Rho   --------------MKDVSISSLENMKLKELYELARHYKISYYSKLTKKELIFAILKANA
E. coli Rho   ----------------MNLTELKNTPVSELITLGENMGLENLARMRKQDIIFAILKQHA

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EKDGNYYMEGILDDIQPDGYGFLRTVN--YSKGEKDIYISASQIRRFEIKRGDKVTGKVR
EQEDLLFMEGVLEIIQSEGFGFLRPIN--YSPSSEDIYISASQIRRFDLRNGDKVSGKVR
KSGEDIFGDGVLEILQ-DGFGFLRSADSSYLAGPDDIYVSPSQIRRFNLRTGDTISGKIR

KPKDNEKYYGLLQVDFVNDHNAEEVKKRPHFQALTPLYPDERIKLET---ETQNYSTRIM
PPKENERYYGLLHVEAVNGDDPESAKERVHFPALTPLYPDRQMVLET---KPNFLSTRIM
PPKEGERYFALLKVNEVNFDKPENARNKILFENLTPLHANSRLRMERGNGSTEDLTARVL

DLVTPIGLGQRGLIVAPPKAGKTSLLKEIANAISTNKPDAKLFILLVGERPEEVTDLERS
DMMAPVGFGQRGLIVAPPKAGKTMLLKEIANSITANQPEAELIVLLIDERPEEVTDIERS
DLASPIGRGQRGLIVAPPKAGKTMLLQNIAQSIAYNHPDCVLMVLLIDERPEEVTEMQRL

VEAAEVVHSTFDEPPEHHVKVAELLLERAKRLVEIGEDVIILMDSITRLARAYNLVIPPS
VAG-DVVSSTFDEVPENHIKVAELVLERAMRLVEHKKDVIILMDSITRLARAYNLVIPPS
VKG-EVVASTFDEPASRHVQVAEMVIEKAKRLVEHKKDVIILLDSITRLARAYNTVVPAS

GRTLSGGLDPCILHKPKAFFGAARNIEAGGSLTILATALVDTGSRMDDMIYEEFKGTGNM
GRTLSGGIDPAAFHRPKRFFGAARNIEEGGSLTILATALVDTGSRMDDVIYEEFKGTGNM
GKVLTGGVDANALHRPKRFFGAARNVEEGGSLTIIATALIDTGSKMDEVIYEEFKGTGNM

ELHLDRKLSERRIFPAIDIGRSSTRKEELLISKSELDTLWQLRNLFT--DSTDFTERFIR
ELHLDRSLAERRIFPAIDIRRSGTRKEELLVPKEHLDRLWSIRKTMS--DSPDFAEKFMR
ELHLSRKIAEKRVFPAIDYNRSGTRKEELLTTQEELQKMWILRKIIHPMGEIDAMEFLIN

KLKRSKNNEDFFKQLQKSAEESTKTGRPII
KMKKTKTNQEFFDILNQEWKQANLSSARR-
KLAMTKTNDDFFEMMKRS-----------
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FIG. 2. Alignment of *S. aureus* Rho with *B. subtilis* and *E. coli* Rho. Sequences were aligned by using the MegAlign program of the DNAstar DNA analysis package. Identical residues are shaded.

hundred colonies isolated from the *rho* mutant infection were checked for tetracycline resistance, indicative of the presence of the mutation, and all were found to be tetracycline resistant.

Hematogenous pyelonephritis infections. Six- to eight-week-old male CD-1 mice were used for hematogenous pyelonephritis infections. Cultures of WCUH29 and RSW101 cells were adjusted to an A_{600} of 0.6 or 0.3 per ml, and 200 µl was injected into each mouse via a tail vein. Mice were sacrificed on day 5 postinfection by CO_2 overdose, and their kidneys were aseptically removed and then homogenized in 1 ml of PBS in a stomacher, and viable counts were enumerated (described above). The inoculum for both strains was 10^7 cells. The number of bacteria recovered from the kidneys infected with RSW101 after 5 days was not decreased. One hundred colonies from the RSW101 infection were checked for the tetracycline resistance marker, and 98% were found to be tetracycline resistant.

Nucleotide sequence accession number. The nucleotide sequence of the *S. aureus rho* gene has been deposited in GenBank under accession no. AF333962.

RESULTS

The *S. aureus* Rho protein. *S. aureus* Rho is predicted to code for a 443-amino-acid protein with a predicted molecular mass of 50 kDa. *S. aureus* Rho is 53% identical and 67% similar to *E. coli* Rho and 66% identical and 75% similar to *B. subtilis* Rho (Fig. 2). The predicted ATPase domain (residues 167 to 342 of *E. coli* Rho) is highly conserved among different bacterial species (18). The predicted ATPase domain of *S. aureus* Rho (residues 180 to 356) is 80% similar and 70% identical to *E. coli* Rho and 86% similar and 81% identical to *B. subtilis* Rho. Finally, the predicted RNA binding domain of *S. aureus* Rho (residues 40 to 132) has 63% similarity and 41% identity to *E. coli* Rho and 77% similarity and 60% identity to *B. subtilis* Rho.

Complementation. The *S. aureus rho* gene can complement the lack of growth of an *E. coli* strain with *rho* deleted.. This was determined by curing a *rho*-deleted strain of a plasmid that carries *E. coli rho* in the presence of *S. aureus rho* carried on another plasmid. The sole Rho activity in the resulting strain, BLS107(pAU2442), is from *S. aureus* Rho. Strain BLS107 (pAU2442) is sensitive to bicyclomycin, as indicated by the bicyclomycin MIC of 125 µg/ml, indicating that *S. aureus* Rho is inhibited by bicyclomycin. As further evidence of this, bicyclomycin was found to inhibit the ATPase activity of partially purified *S. aureus* Rho (data not shown).

The *S. aureus rho* gene is not essential for in vitro growth. A Δ*rho* strain, RSW100, was constructed by replacing nearly all of the *rho* gene with a tetracycline resistance cassette (Fig. 1). The resulting strain is viable and grows normally at 30, 37, 42,

TABLE 2. Bacterial phyla with members that
possess a Rho homolog[a]

Aquificales
  Aquifex aeolicus

Chlamydiales
  Chlamydia muridarum
  Chlamydia pneumoniae
  Chlamydia trachomatis

Cytophaga-Flexibacter-Bacteroides
  Porphyromonas gingivalis

Green sulfur
  Chlorobium tepidum

Gram positive
  Low G+C (not *Streptococcus pyogenes, Mycoplasma pneumoniae,*
    Mycoplasma genitalium, or *Streptococcus pneumoniae*)
    Bacillus subtilis
    Clostridium acetobutylicum
    Listeria monocytogenes

  High G+C (*Actinobacteria*)
    Micrococcus luteus
    Mycobacterium leprae
    Mycobacterium tuberculosis
    Streptomyces coelicolor
    Streptomyces lividans

Proteobacteria
  α Subdivision
    Rhodobacter sphaeroides
    Rickettsia prowazekii

  β Subdivision
    Neisseria animalis
    Neisseria cinerea
    Neisseria elongata
    Neisseria gonorrhoeae
    Neisseria lactamica
    Neisseria meningitidis
    Neisseria mucosa
    Neisseria pharyngis
    Neisseria polysaccharea
    Neisseria subflava

  ε Subdivision
    Campylobacter jejuni
    Helicobacter pylori

  γ Subdivision
    Actinobacillus actinomycetemcomitans
    Actinobacillus pleuropneumoniae
    Allochromatium vinosum
    Buchnera aphidicola
    Escherichia coli
    Haemophilus influenzae
    Pseudomonas aeruginosa
    Pseudomonas fluorescens
    Salmonella enterica serovar Typhimurium
    Vibrio cholerae
    Xylella fastidiosa
    Yersinia pestis

Spirochaetales
  Borrelia burgdorferi
  Treponema pallidum

Thermotogales
  Thermotoga maritima

Thermus/Deinococcus
  Deinococcus radiodurans

  [a] Groupings taken from http://www.ncbi.nih.gov/htbin-post/Taxonomy/wgetorg
and determined by BLAST analysis with *S. aureus* Rho.

and 46°C. RSW100 also grows normally on rich medium (LB or tryptic soy broth) or minimal medium (supplemented M9 medium) and under anaerobic conditions (data not shown).

rho is not required for S. aureus virulence. The *rho* deletion was transduced into the pathogenic clinical isolate WCUH29, yielding strain RSW101. Cultures of WCUH29 and RSW101 were used in the two in vivo models described in Materials and Methods. *S. aureus* strains mutated in a variety of different genes affecting cell metabolism or virulence have been examined in our laboratories. These strains have displayed reductions of up to 3 logs in the pyelonephritis model and 1 log in the surgical wound infection. In the experiments described here, the in vivo growth of RSW101 was not impaired compared to that of wild-type WCUH29 in either of the model systems. From these data, we conclude that, as with in vitro growth, *rho* is not essential for the virulence of the strain tested.

Review of Rho homologs present in public sequence database. By using the *S. aureus* Rho sequence as a query, we searched the public databases and some proprietary sequence databases for homologs. Rho was found to be highly conserved; BLAST scores were 5×10^{-38} or lower for all full-length Rho sequences annotated as such. As indicated previously by Opperman and Richardson (18), Rho is broadly distributed among all the major phyla of the eubacteria (Table 2). A major exception to the universal presence of Rho among the eubacteria are the members of the low-GC, gram-positive bacteria *Streptococus pyogenes*, *Streptococcus pneumoniae*, *Mycoplasma genitalium*, *M. pneumoniae*, and *Ureaplasma urealyticum,* all of which do not possess a significant homolog. In the case of *S. pneumoniae*, we searched several proprietary databases in addition to publicly available databases and failed to identify a homolog. In addition to the mentioned bacteria, *Synechocystis* sp. strain PCC6803, a cyanobacterium, also apparently lacks a Rho homolog. As indicated previously (18), the ATP binding motif of Rho shares homology with the α and β subunits of F1 ATPases. This homology is conserved among the bacteria as well.

DISCUSSION

In this report, we show that the *S. aureus rho* gene can complement an *E. coli rho* deletion strain. The ability of *S. aureus rho* to replace the essential *E. coli rho* gene raises the possibility that it performs the same functions in *S. aureus* and *E. coli*. *rho* is not essential in the low-GC, gram-positive bacterium *Bacillus subtilis* (13, 21), and several gram-positive species, including *Staphylococcus aureus*, are resistant to the Rho inhibitor bicyclomycin (15). Here we show that *rho* is not required for viability or virulence in *S. aureus*. The *E. coli* strain constructed here, which has had its own *rho* gene replaced with *S. aureus rho*, remains sensitive to bicyclomycin. This suggests that the resistance of gram-positive bacteria to bicyclomycin is a result of the nonessentiality of Rho. Supporting this conclusion and extending the lack of essentiality of *rho* to other genera, we found that the completed genomes of several members of the low-GC, gram-positive bacteria lack a Rho homolog. The cyanobacterium *Synechocystis* sp. strain PCC6803 was also found to lack a Rho homolog.

An open question is why *rho* should be essential in *E. coli*

and other gram-negative bacteria, but not be essential in some gram-positive bacteria. It could be that Rho activity is redundant in the bacteria where it is not essential. This possibility cannot be readily discounted, but it is clear that the enzyme responsible for this redundant activity would have to have no sequence homology with Rho for this to be the case. A second possibility, that Rho is essential for organisms with a higher GC content, seems unlikely, given that *Synechocystis* sp. strain PCC6803 also lacks Rho, and yet its genome is 47% GC, close to that of *E. coli*. The lack of *rho* among several members of the low-GC, gram-positive bacteria and a member of the very distantly related cyanobacteria could indicate a trend that the essentiality of *rho* may be more the exception than the rule. Because of this, a more informative approach to the question is to ask why *rho* is essential in *E. coli* and other gram-negative bacteria. There are only a few Rho-dependent terminators in *E. coli*; the vast majority of *E. coli* operons contain factor-independent terminators. Furthermore, it is not obvious why inhibition of termination at Rho-dependent terminators would lead to a lethal event. It might be that there is some other function of Rho that better explains its essential nature in some bacteria.

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