JOURNAL OF BACTERIOLOGY, Dec. 2005, p. 8205–8210 Vol. 187, No. 23
0021-9193/05/$08.00+0 doi:10.1128/JB.187.23.8205–8210.2005

Vancomycin Stress Response in a Sensitive and a Tolerant Strain of *Streptococcus pneumoniae*

Wolfgang Haas,[1]* Deepak Kaushal,[2] Jack Sublett,[1] Caroline Obert,[1] and Elaine I. Tuomanen[1]

Departments of Infectious Diseases[1] and Functional Genomics,[2] Hartwell Center for Bioinformatics and Biotechnology, St. Jude Children's Research Hospital, Memphis, Tennessee

Received 13 July 2005/Accepted 15 September 2005

The vancomycin stress response was studied in *Streptococcus pneumoniae* strains T4 (TIGR4) and Tupelo. Vancomycin affected the expression of 175 genes, including genes encoding transport functions and enzymes involved in aminosugar metabolism. The two-component systems TCS03, TCS11, and CiaRH also responded to antibiotic treatment. We hypothesize that the three regulons are an important part of the bacterium's response to vancomycin stress.

A considerable number of strains of *Streptococcus pneumoniae*, the causative agent of pneumonia, bacteremia, otitis media, and meningitis, have become resistant to commonly used antibiotics. This has prompted the increased use of vancomycin, especially in cases of sepsis and meningitis, since no vancomycin-resistant isolates of *S. pneumoniae* have been reported to date. Vancomycin-sensitive strains stop growing in the presence of the antibiotic and rapidly undergo cell death through autolysis. Vancomycin-tolerant isolates have been described that cease to grow but do not undergo a significant degree of autolysis and retain the ability to grow once the antibiotic has dissipated (2, 10, 11, 17). This phenotype has been linked to treatment failure (15) and could foster the development of resistant strains that are able to not only survive but also grow in the presence of vancomycin. The recent emergence of vancomycin resistance in enterococci and staphylococci (1, 7, 22) is further cause for concern in this regard.

To gain insight into the effect of vancomycin on transcription in pneumococcus, we compared the stress response to vancomycin in two clinical isolates. The sequenced strain T4 (serotype 4) (21) is vancomycin sensitive, while strain Tupelo (serotype 14) is naturally vancomycin tolerant (17). Unlike T4, Tupelo does not undergo autolysis during stationary phase and lyses much slower in response to vancomycin, despite the presence of a fully functional LytA autolysin (17).

Experimental design. Both strains were grown in C+Y medium to mid-logarithmic growth phase (optical density at 620 nm, 0.45 to 0.5) and exposed to 5 μg/ml vancomycin (equal to the 10-fold vancomycin MICs for each strain) for 10 and 20 min. The latter time point was chosen because it coincided with the cessation of growth and the onset of autolysis. RNA isolation and microarray analyses were performed as described previously (9). cDNA microarrays specific for strain T4 were received as a grant from the Pathogen Functional Genomics Resource Center (PFGRC; The Institute for Genomic Research, Rockville, MD). Three independent biological samples were used for each experiment. Genes that were differentially regulated by more than threefold and that had an analysis of variance *P* value of 0.001 or lower were considered further. The complete set of microarray data can be downloaded from St. Jude's web site (http://www.stjuderesearch.org/data/VancoT4Tupelo/).

Common themes in vancomycin stress response in strains T4 and Tupelo. A number of transcripts exhibited similar expression patterns in both strains after vancomycin treatment. The *hrcA-grpE-dnaK-dnaJ* operon, encoding heat shock proteins, was induced in response to vancomycin in both strains, although expression levels fell to basal levels in strain T4 after 20 min (Table 1). The expression of other stress response genes, such as *gor* and *htpX*, increased in strain T4 by 3- to 6-fold in T4 but only 1.7- to 2.0-fold in Tupelo. A transcriptional regulator of the GntR family and the two-component systems TCS03 and TCS11 (13) were induced in both strains as well (see below). The choline binding proteins G and F were induced three- to fourfold within 10 min of vancomycin treatment. Other genes that were induced in both strains include a number of ABC transporters of unknown substrate specificity (i.e., SP1380/1, SP1715, and SP2003) and hypothetical proteins (i.e., SP0099, SP0385, and SP0910). Genes involved in aminosugar metabolism also responded in both strains: the *glmS* gene product catalyzes the synthesis of aminosugars, while the NagA and NagB proteins play a role in their catabolism. The expression of *glmS* was up to 20-fold reduced in vancomycin-treated T4 cultures, while *nagA* and *nagB* expression increased 15- and 18-fold, respectively, under the same conditions. In strain Tupelo, the genes followed the same overall expression pattern, although changes in transcription were only two- to fourfold. The expression of ribosomal proteins and translation factors decreased after the addition of the antibiotic, especially in strain T4. Transcripts of genes that play a role in the metabolism of nitrogen, polyamine, and purines were reduced in their expression as well.

Responses to vancomycin stress which are unique to strain T4 or Tupelo. While some genes responded to vancomycin treatment similarly in both strains, other genes were induced or repressed in one strain but not the other. Expression of the *cps4* genes, which are responsible for synthesis of the type 4 capsule, and of a locus that encodes three cell wall surface anchor family proteins was reduced in strain T4. No significant

* Corresponding author. Mailing address: Department of Microbiology and Immunology, Center for Oral Biology, University of Rochester Medical Center, 601 Elmwood Ave., Rochester, NY 14642. Phone: (585) 275-7722. Fax: (585) 276-0190. E-mail: wolfgang_haas@urmc.rochester.edu.

TABLE 1. Genes that are differentially expressed in *S. pneumoniae* strains T4 and Tupelo 10 and 20 min after the addition of vancomycin[a]

Identity and function	Annotation	Gene	T4 with vancomycin for:		Tupelo with vancomycin for:	
			10 min	20 min	10 min	20 min
Stress response						
SP0515	Heat-inducible transcription repressor HrcA	*hrcA*	5.8	−1.0 s	4.7	3.3
SP0516	Heat shock protein GrpE	*grpE*	6.1	1.2 s	4.8	3.1
SP0517	DnaK protein	*dnaK*	6.6	2.0	4.6	3.5
SP0519	DnaJ protein	*dnaJ*	3.9	1.6 s	3.8	3.1
SP0766	Superoxide dismutase, manganese dependent	*sodA*	1.3 s	1.3 s	−1.1 s	−3.4
SP0784	Glutathione reductase	*gor*	2.0	3.3	1.6 s	1.9
SP1283	Heat shock protein HtpX	*htpX*	1.7	3.5	1.4 s	1.7
SP1284	LemA protein	*lemA*	1.7	3.5	1.5 s	1.8
SP1996	Universal stress protein family	*uspA*	2.1	6.0	−1.6 s	−2.5
SP2206	Ribosomal subunit interface protein	*yfiA*	1.7 s	3.3	−1.7 s	−2.7
Transcription factors and two-component systems						
SP0386	Sensor histidine kinase	*HK03*	5.3	5.5	4.1	5.3
SP0387	DNA-binding response regulator	*RR03*	4.7	4.4	4.4	5.1
SP0727	Transcriptional repressor, putative		1.4 s	4.3	1.4 s	1.3 s
SP1714	Transcriptional regulator, GntR family		6.8	27.6	9.8	8.1
SP2000	DNA-binding response regulator	*RR11*	2.7	2.5	3.6	3.3
SP2001	Sensor histidine kinase	*HK11*	3.4	2.8	3.8	3.7
Aminosugar and cell wall metabolism						
SP0266	Glucosamine-fructose-6-phosphate aminotransferase	*glmS*	−8.3	−20.1	−4.2	−3.9
SP1415	Glucosamine-6-phosphate isomerase	*nagB*	7.8	18.5	3.0	2.0
SP1975	SpolllJ family protein		2.3	2.3	2.6	3.1
SP2056	*N*-Acetylglucosamine-6-phosphate deacetylase	*nagA*	7.1	15.6	2.6	2.4
SP2217	Rod shape-determining protein MreD, putative		−1.5 s	−3.1	−1.0 #s	−1.1 s
SP2218	Rod shape-determining protein MreC	*mreC*	−1.4 s	−2.6	1.1 #s	1.1 s
Nitrogen, purine, and polyamine metabolism						
SP0044	Phosphoribosylaminoimidazole-succinocarboxamide synthase	*purC*	−2.7	−3.5	1.1 s	−6.5
SP0045	Phosphoribosylformylglycinamidine synthase, putative		−2.0 s	−3.1	1.2 s	−3.7
SP0231	Adenylate kinase	*adk*	−1.8 s	−5.1	−1.6 s	−1.6 s
SP0287	Xanthine/uracil permease family protein		−2.4	−6.2	−1.3 s	−3.1
SP0502	Glutamine synthetase, type I	*glnA*	−2.2	−3.3	−1.4 s	−1.4 s
SP0916	Lysine decarboxylase	*cad*	−2.5	−6.9	−1.7 #s	−1.2 #s
SP0918	Spermidine synthase	*speE*	−2.4	−7.8	−2.4	−2.1 s
SP0920	Carboxynorspermidine decarboxylase	*nspC*	−1.9 s	−7.2	−2.1 s	−2.2
SP0922	Carbon-nitrogen hydrolase family protein		−2.0	−7.0	−2.1 s	−2.2
SP1180	Ribonucleoside-diphosphate reductase 2, beta subunit	*nrdF*	−1.7	−3.4	−1.3 s	−1.7
SP1847	Xanthine phosphoribosyltransferase	*xpt*	−2.3	−10.9	1.2 s	−3.0
SP1848	Xanthine permease	*pbuX*	−2.4	−11.3	1.2 s	−2.3
Fermentation, polysaccharide, and sugar metabolism						
SP0285	Alcohol dehydrogenase, zinc containing		2.3	3.1	−3.4	−3.5
SP0459	Formate acetyltransferase	*pfl*	1.7	1.9	−2.1	−4.2
SP1118	Pullulanase, putative		−1.6 s	−3.9	−1.1 s	−1.1 s
SP1122	Glucose-1-phosphate adenylyltransferase	*glgC*	1.2 s	1.5 s	−2.3 s	−3.2
SP1123	Glycogen biosynthesis protein GlgD	*glgD*	1.2 s	1.3 s	−2.1 s	−3.1
SP1329	*N*-Acetylneuraminate lyase		1.7 #s	1.6 #s	−2.4 s	−4.0
SP1330	*N*-Acetylmannosamine-6-P epimerase, putative	*nanE*	1.4 #s	1.7 #s	−2.7 s	−4.4
SP1382	Alpha-amylase	*amy*	3.1	6.0	1.2 #s	2.1 #s
SP1852	Galactose-1-phosphate uridylyltransferase	*galT*	1.0 s	−1.0 s	−1.9 s	−3.3
SP2026	Alcohol dehydrogenase, iron-containing	*adhE*	2.8	1.4 s	−3.4	−3.2
SP2106	Glycogen phosphorylase family protein		3.3	3.4	−1.0 s	−1.2 s
SP2107	4-Alpha-glucanotransferase	*malQ*	4.3	3.9	−1.0 s	−1.2 s
SP2157	Alcohol dehydrogenase, iron-containing	*fucO*	3.7	5.3	−1.2 #s	−1.2 #s
SP2167	L-Fuculose kinase FucK, putative	*fucK*	2.4 #s	4.1	1.2 #s	1.5 #s
Capsule biosynthesis						
SP0346	Capsular polysaccharide biosynthesis protein Cps4A	*cps4A*	−1.4 s	−1.8	1.0 s	1.1 s

Continued on facing page

TABLE 1—*Continued*

Identity and function	Annotation	Gene	T4 with vancomycin for:		Tupelo with vancomycin for:	
			10 min	20 min	10 min	20 min
SP0347	Capsular polysaccharide biosynthesis protein Cps4B	*cps4B*	−1.3 s	−1.5	1.1 s	1.1 s
SP0348	Capsular polysaccharide biosynthesis protein Cps4C	*cps4C*	−1.5 s	−2.4	−1.0 s	1.1 s
SP0349	Capsular polysaccharide biosynthesis protein Cps4D	*cps4D*	−1.7	−3.1	−1.2 s	−1.2 s
SP0350	Capsular polysaccharide biosynthesis protein Cps4E	*cps4E*	−1.9	−4.0	−1.0 #s	1.2 #s
SP0351	Capsular polysaccharide biosynthesis protein Cps4F	*cps4F*	−1.9	−4.3	1.4 #s	−1.4 #s
SP0352	Capsular polysaccharide biosynthesis protein Cps4G	*cps4G*	−1.9 s	−4.0	1.1 #s	1.1 #s
SP0353	Capsular polysaccharide biosynthesis protein Cps4H	*cps4H*	−1.6	−1.5	1.1 #s	−1.0 #s
SP0357	UDP-*N*-acetylglucosamine-2-epimerase	*cps4I*	−2.0	−6.6	−1.0 #s	−1.0 #s
SP0358	Capsular polysaccharide biosynthesis protein Cps4J	*cap4J*	−1.8 s	−6.3	1.7 #s	1.0 #s
SP0359	Capsular polysaccharide biosynthesis protein Cps4K	*cps4K*	−1.8	−6.4	1.1 #s	−1.3 #s
SP0360	UDP-*N*-acetylglucosamine-2-epimerase	*cps4L*	−1.7	−5.8	1.2 #s	1.2 #s
Surface proteins						
SP0390	Choline binding protein G	*cbpG*	3.9	4.4	3.4	4.3
SP0391	Choline binding protein F	*cbpF*	3.2	4.2	3.0	4.5
SP0462	Cell wall surface anchor family protein		−1.5 s	−2.6	1.0 #s	1.2 #s
SP0463	Cell wall surface anchor family protein		−1.7 s	−3.4	1.5 #s	1.3 #s
SP0464	Cell wall surface anchor family protein		−1.5 s	−3.7	1.1 #s	−1.3 #s
SP1002	Adhesion lipoprotein	*lmb*	−1.8 s	−4.3	1.0 s	−1.3 s
Translation and ribosomal proteins						
SP0085	Ribosomal protein S4	*rpsD*	−2.6	−5.0	−1.6 s	−2.0
SP0232	Translation initiation factor IF-1	*infA*	−1.8	−3.0	−1.5 s	−1.7
SP0233	Ribosomal protein L36	*rpmJ*	−1.8	−2.9	−1.6 s	−1.9 s
SP0234	Ribosomal protein S13	*rpsM*	−1.8	−3.3	−1.5 s	−1.7
SP0235	Ribosomal protein S11	*rpsK*	−1.8	−3.3	−1.6	−1.6
SP0271	Ribosomal protein S12	*rpsL*	−1.8	−3.3	−1.4 s	−1.5 s
SP0272	Ribosomal protein S7	*rpsG*	−1.7	−3.1	−1.4 s	−1.4 s
SP0294	Ribosomal protein L13	*rplM*	−1.8	−3.1	−1.4 s	−1.5
SP0775	Ribosomal protein S16	*rpsP*	−1.7	−3.8	−1.2 s	−2.3
SP0862	Ribosomal protein S1	*rpsA*	−2.2	−3.5	−1.4 s	−1.8
SP0959	Translation initiation factor IF-3	*infC*	−1.8	−4.9	−1.3 s	−1.1 s
SP0960	Ribosomal protein L35	*rpmI*	−1.6 s	−3.2	−1.3 s	−1.4 s
SP0961	Ribosomal protein L20	*rplT*	−1.8	−3.2	−1.4 s	−1.5 s
SP1354	Ribosomal protein L7/L12	*rplL*	−1.6	−3.0	−1.6 s	−1.6
SP1355	Ribosomal protein L10	*rplJ*	−1.5	−3.3	−1.5 s	−1.2 s
SP2214	Translation elongation factor Ts	*tsf*	−1.2	−3.2	−1.2 s	−1.3 s
SP2215	Ribosomal protein S2	*rpsB*	−1.3	−3.8	−1.2 s	−1.3 s
PTS systems and ABC transporters						
SP0090	ABC transporter, permease protein		3.2	5.2	1.3 #s	1.6 #s
SP0091	ABC transporter, permease protein		2.0 s	3.1	1.2 #s	−1.1 #s
SP0092	ABC transporter, substrate-binding protein		2.9	4.8	1.4 #s	1.3 #s
SP0282	PTS system, mannose-specific IID component	*manN*	−1.2	−1.3	−2.2 s	−3.4
SP0283	PTS system, mannose-specific IIC component	*manM*	−2.6	−3.2	−2.3	−3.0
SP0284	PTS system, mannose-specific IIAB components	*manL*	−2.8	−3.8	−1.9 s	−3.3
SP0786	ABC transporter, ATP-binding protein		2.0	3.0	3.2	2.2
SP0912	ABC transporter, ATP-binding protein		3.2	5.5	1.8 s	1.9 s
SP0913	ABC transporter, permease protein, putative		3.1	5.0	1.8 s	2.2
SP0957	ABC transporter, ATP-binding protein		1.2 s	1.2 s	−1.9 s	−3.1
SP1032	Iron compound ABC transporter, iron compound-binding protein	*piuB*	−1.5 s	−2.3	−1.2 s	−1.3 s
SP1033	Iron compound ABC transporter, permease protein	*piuC*	−2.0 s	−4.3	1.1 #s	1.1 s
SP1034	Iron compound ABC transporter, permease protein	*piuD*	−2.0 s	−4.6	−1.1 #s	1.1 s
SP1035	Iron compound ABC transporter, ATP-binding protein	*piuA*	−2.0 s	−4.6	−1.1 s	−1.1 s
SP1380	Putative permease		2.0	3.0	2.1	3.3
SP1381	ABC transporter, ATP-binding protein		2.4 s	5.8	2.0 #s	3.3
SP1580	Sugar ABC transporter, ATP-binding protein	*msmK*	−1.1 s	−1.1 s	−3.5	−6.6
SP1688	ABC transporter, permease protein		1.8 s	3.8	1.2 #s	−1.5 #s
SP1689	ABC transporter, permease protein		2.0 s	2.9	1.1 #s	−1.1 #s
SP1690	ABC transporter, substrate-binding protein		2.1 s	3.6	1.5 #s	1.6 #s
SP1715	ABC transporter, ATP-binding domain and permease		7.9	27.2	9.7	12.2

Continued on following page

TABLE 1—*Continued*

Identity and function	Annotation	Gene	T4 with vancomycin for:		Tupelo with vancomycin for:	
			10 min	20 min	10 min	20 min
SP2002	Putative permease		4.5	4.4	1.8 #s	1.7 #s
SP2003	ABC transporter, ATP-binding protein		10.3	9.2	4.4	4.9
SP2108	Maltose/maltodextrin ABC transporter, maltose-binding protein	*malX*	1.7	2.6	−2.7	−4.0
SP2109	Maltodextrin ABC transporter, permease protein	*malC*	1.6 s	2.1	−2.6 s	−3.9
SP2110	Maltodextrin ABC transporter, permease protein	*malD*	1.6 s	2.4	−1.5 #s	−2.5
ClaRH regulon						
SP0282	PTS system, mannose-specific IID component	*manN*	−1.2	−1.3	−2.2 s	−3.4
SP0283	PTS system, mannose-specific IIC component	*manM*	−2.6	−3.2	−2.3	−3.0
SP0284	PTS system, mannose-specific IIAB components	*manL*	−2.8	−3.8	−1.9 s	−3.3
SP0798	DNA-binding response regulator CiaR	*ciaR*	2.7	2.5	−1.1 s	−1.3 s
SP0799	Sensor histidine kinase CiaH	*ciaH*	2.8	2.2	−1.1 s	−1.2
SP0879	Hypothetical protein		9.9	14.1	1.5 #s	1.2 s
SP1027	Conserved hypothetical protein		6.1	9.7	1.2 s	−1.2 s
SP2206	Ribosomal subunit interface protein	*yfiA*	1.7 s	3.3	−1.7 s	−2.7
SP2239	HtrA serine protease	*htrA*	5.4	4.1	−1.2 s	−1.7
SP2240	SpoJ protein	*parB*	4.7	3.6	−1.1 s	−1.6
Miscellaneous functions						
SP0006	Transcription-repair coupling factor	*mfd*	−1.6 s	−3.9	−1.1 s	−1.3 s
SP0109	Bacteriocin, putative		1.9 s	3.5	−1.3 s	−1.6
SP0356	O-antigen transporter RfbX, putative		−1.8 s	−6.5	1.8 #	−1.0 #s
SP0962	Lactoylglutathione lyase	*gloA*	−1.8 s	−3.6	−1.3 s	−1.3
SP1117	DNA ligase, NAD dependent	*ligA*	−1.8 s	−4.3	1.0 s	−1.0 s
SP1214	Transulfuration enzyme family protein, authentic point mutation		2.5 s	3.3	1.0 #s	1.3 #s
SP1325	Oxidoreductase, Gfo/Idh/MocA family		1.9 #s	1.8 #	−2.3 s	−4.0
SP1326	Neuraminidase, putative		1.3 #s	2.1 #s	−2.6 s	−3.7
SP1328	Sodium:solute symporter family protein		1.4 s	1.4 #s	−2.1 s	−3.2
SP1343	Prolyl oligopeptidase family protein		1.9 s	3.2	1.5 #s	1.4 #s
SP1402	NOL1/NOP2/sun family protein		−2.1 s	−4.4	−1.1 s	−1.7 s
SP1466	Hemolysin		2.8	6.1	−1.4 s	−2.5
SP1513	ATP synthase F0, A subunit	*atpB*	1.2 s	1.3 s	1.8 s	3.2
SP1586	ATP-dependent RNA helicase, putative		−1.3 s	−4.4	1.4 s	1.2 s
SP1687	Neuraminidase B	*nanB*	2.1 s	3.4	−1.1 #s	1.4 #s
SP1807	Acetyltransferase, GNAT family		2.1 s	3.3	1.3 #s	−1.1 s
Hypothetical proteins						
SP0034	Membrane protein		1.6	1.7 s	2.2 s	3.9
SP0088	Hypothetical protein		1.5 s	3.2	1.4 #s	1.1 #s
SP0096	Hypothetical protein		1.8 s	3.2	2.0 #s	1.6 #s
SP0097	Conserved domain protein		2.0	2.3	3.5	2.3 s
SP0098	Hypothetical protein		3.2	2.9	3.5	2.6
SP0099	Hypothetical protein		3.6	3.5	3.8	2.9
SP0100	Conserved hypothetical protein		3.4	3.6	3.7	2.3
SP0189	Conserved hypothetical protein		2.9	5.4	2.1	1.7 s
SP0191	Hypothetical protein		3.3	4.0	2.3 s	2.8
SP0288	Conserved hypothetical protein		−3.2	−5.4	−1.0 #s	−1.6 #s
SP0293	Hypothetical protein		2.2	3.5	1.5 s	1.9
SP0298	Conserved hypothetical protein		2.2 s	3.1	1.4 #s	−1.1 #s
SP0355	Hypothetical protein		−2.0	−6.4	1.2 #s	1.0 #s
SP0385	Conserved hypothetical protein		5.5	5.9	3.9	5.1
SP0389	Hypothetical protein		3.4	4.0	2.8 s	3.6
SP0430	Hypothetical protein		−1.5 s	−3.3	−1.3 s	−1.6 s
SP0595	Hypothetical protein		1.0 s	−3.1	1.1 #s	1.8 #s
SP0728	Hypothetical protein		1.5 s	3.6	1.3 s	1.2 s
SP0742	Conserved hypothetical protein		1.9 s	1.6 s	−2.2	−3.7
SP0785	Conserved hypothetical protein		2.2	3.0	3.3	2.4
SP0787	Conserved hypothetical protein		2.0 s	3.2	3.5	2.2
SP0879	Hypothetical protein		9.9	14.1	1.5 #s	1.2 s
SP0910	Conserved hypothetical protein		4.1	8.0	3.2	3.4
SP0919	Conserved hypothetical protein		−2.3 s	−6.0	−1.9 s	−2.0 #
SP0921	Conserved hypothetical protein		−1.9 s	−6.9	−2.3	−2.3
SP0925	Conserved hypothetical protein		1.7 s	3.2	1.5 #s	1.9 s
SP0958	Hypothetical protein		1.1 s	1.1 s	−2.3	−4.1

Continued on facing page

TABLE 1—*Continued*

Identity and function	Annotation	Gene	T4 with vancomycin for:		Tupelo with vancomycin for:	
			10 min	20 min	10 min	20 min
SP1004	Conserved hypothetical protein		−1.3 s	−4.0	2.4 s	1.3
SP1027	Conserved hypothetical protein		6.1	9.7	1.2 s	−1.2 s
SP1327	Conserved hypothetical protein		1.3 #s	1.4 #s	−2.3 s	−3.6
SP1344	Conserved hypothetical protein		3.1 #s	4.9	1.7 #s	1.2 #s
SP1465	Hypothetical protein		3.2	7.3	−1.4 s	−2.9
SP1532	Conserved domain protein, authentic frameshift		−1.7 s	−3.8	−1.1 s	−1.2 s
SP1612	Conserved domain protein		1.2 #s	−1.2 s	1.3 #s	3.2
SP1685	Conserved hypothetical protein		1.1 s	1.5 s	−2.2	−3.3
SP1691	Conserved hypothetical protein		1.8 #s	5.4	1.4 #s	2.2 s
SP1716	Conserved hypothetical protein	*natB*	3.3	4.2	1.0 s	1.1 s
SP1972	Membrane protein		2.7	3.4	1.2 s	1.1 s
SP2004	Hypothetical protein		4.6	4.3	1.7 #s	1.3 #s
SP2005	Hypothetical protein		6.6	9.5	1.8 #s	1.1 #s

[a] Data represent fold increase or decrease in gene expression after treatment. Only genes whose expression changed by at least threefold are shown. Each datum point represents three biological samples and a total of 12 cDNA hybridization spots. A "#" marks data that are based on low signal intensities for the sample and the reference. Data that had analysis of variance *P* values larger than 0.001 are indicated by an "s."

signal was obtained in the case of strain Tupelo, because the loci are either divergent or missing (data not shown). The expression of stress response genes, such as *uspA*, *yfiA*, and *adhE*, increased in T4 but decreased in Tupelo. Expression of superoxide dismutase decreased by 3.4-fold in Tupelo but remained steady in T4. The *pfl* and *adhE* genes, whose products are involved in mixed acid fermentation, as well as two other genes encoding alcohol dehydrogenases were induced in strain T4 but remained unchanged or were repressed in Tupelo. Transcripts for glycolytic enzymes were increased in strain T4, while the expression of genes involved in glycogen biosynthesis was decreased in Tupelo. Several ABC transporters and hypothetical proteins were also differentially expressed in one strain but not the other.

A significant difference in gene expression was observed for the CiaRH regulon. The CiaRH two-component system has been shown to regulate various functions in *S. pneumoniae*, such as autolysis, competence, virulence, and beta-lactam susceptibility (5, 8, 20, 24). Several screens have identified a number of genes that could be regulated by the CiaRH system (15, 19). Some of these were differentially regulated in response to vancomycin, including the *manLMN* mannose-specific phosphotransferase (PTS) system, the *ciaRH* two-component system itself, the hypothetical proteins SP0879 and SP1027, the iron compound ABC transporter *piuBCDA*, the two-component system TCS11, the ribosomal subunit interface protein YfiA, the serine protease HtrA, and the Spo0J-like protein ParB. Most of the genes listed above are up-regulated (or derepressed) in vancomycin-treated cultures of strain T4 but down-regulated or not differentially expressed in strain Tupelo.

Similarities to the vancomycin stress response in *Staphylococcus aureus* and *Bacillus subtilis*. Work with *S. aureus* has shown that the VraSR two-component system is upregulated in response to treatment with vancomycin and other inhibitors of cell wall synthesis (12, 18). In *B. subtilis*, exposure to vancomycin results in the activation of alternate sigma factors and two-component systems, including LiaRS (YvqCE) (16). A BLASTP search revealed that the pneumococcal two-component system TCS03 (SP0386 and SP0387), which was induced in T4 and Tupelo after vancomycin treatment, has significant similarity to VraSR and to LiaRS. The histidine kinases share 38 to 40% identical and 62 to 65% similar residues with HK03, while the response regulators share 51% identical and 73 to 76% conserved amino acids with RR03. The loci encoding the two-component systems in the three species are preceded by predicted membrane proteins that share 24 to 29% identical and 51 to 53% conserved residues. The three proteins are 232 to 241 amino acids in size and contain the conserved domain COG1458 (14).

The HtrA serine protease, which is part of the CiaRH regulon in pneumococcus, was also induced in all three bacterial species in response to vancomycin stress (4, 12), although the corresponding gene was not induced in *S. pneumoniae* strain Tupelo.

A protein with similarity to phage shock protein A, LiaH (YvqH), has been shown to play a role in the vancomycin stress response in *B. subtilis* (12). In *S. pneumoniae*, the open reading frame SP0910 is induced by vancomycin and encodes a conserved hypothetical protein that contains a phage shock protein C domain. The *pspABCDE* operon from *Escherichia coli* is induced in response to ethanol, heat, osmotic shock, and bacteriophage infection (3). Phage shock proteins A and C play a role in the repression and activation (6, 23) of stress-responsive genes, respectively. Whether the SP0910 gene product has a similar function in *S. pneumoniae* remains to be determined.

Conclusions. The data presented here demonstrate that the vancomycin-sensitive strain T4 and the vancomycin-tolerant strain Tupelo have a number of genes in common that are differentially expressed in response to vancomycin stress. The two-component systems TCS03 and TCS11 were induced in both strains, of which the former shares sequence similarity with a vancomycin-induced two-component system from *S. aureus* and *B. subtilis*. Genes that responded to vancomycin in one pneumococcal strain but not the other were also observed in large numbers. The CiaRH regulon, which has been shown to play a role in autolysis, was induced in strain T4 but not Tupelo. It will be interesting to ascertain if lack of induction of this regulon is the reason for the tolerant phenotype of strain Tupelo.

This work was supported by grant 5R01AI039482-07 from the National Institute of Allergy and Infectious Diseases, Cancer Center grant P30 CA21765, and the American Lebanese Syrian Associated Charities.

REFERENCES

1. **Boneca, I. G., and G. Chiosis.** 2003. Vancomycin resistance: occurrence, mechanisms and strategies to combat it. Expert Opin. Ther. Targets **7:**311–328.
2. **Boost, M. V., W. M. Ko, and M. M. O'Donoghue.** 2003. Penicillin and vancomycin tolerance among clinical isolates of *Streptococcus pneumoniae* in Hong Kong. Hong Kong Med. J. **9:**415–418.
3. **Brissette, J. L., L. Weiner, T. L. Ripmaster, and P. Model.** 1991. Characterization and sequence of the *Escherichia coli* stress-induced *psp* operon. J. Mol. Biol. **220:**35–48.
4. **Cao, M., T. Wang, R. Ye, and J. D. Helmann.** 2002. Antibiotics that inhibit cell wall biosynthesis induce expression of the *Bacillus subtilis* sigma(W) and sigma(M) regulons. Mol. Microbiol. **45:**1267–1276.
5. **Dagkessamanskaia, A., M. Moscoso, V. Henard, S. Guiral, K. Overweg, M. Reuter, B. Martin, J. Wells, and J. P. Claverys.** 2004. Interconnection of competence, stress and CiaR regulons in *Streptococcus pneumoniae*: competence triggers stationary phase autolysis of *ciaR* mutant cells. Mol. Microbiol. **51:**1071–1086.
6. **Elderkin, S., P. Bordes, S. Jones, M. Rappas, and M. Buck.** 2005. Molecular determinants for PspA-mediated repression of the AAA transcriptional activator PspF. J. Bacteriol. **187:**3238–3248.
7. **Endtz, H. P., B. N. van den, H. A. Verbrugh, and A. van Belkum.** 1999. Vancomycin resistance: status quo and quo vadis. Eur. J. Clin. Microbiol. Infect. Dis. **18:**683–690.
8. **Guenzi, E., A. M. Gasc, M. A. Sicard, and R. Hakenbeck.** 1994. A two-component signal-transducing system is involved in competence and penicillin susceptibility in laboratory mutants of *Streptococcus pneumoniae*. Mol. Microbiol. **12:**505–515.
9. **Haas, W., J. Sublett, D. Kaushal, and E. I. Tuomanen.** 2004. Revising the role of the pneumococcal *vex-vncRS* locus in vancomycin tolerance. J. Bacteriol. **186:**8463–8471.
10. **Henriques, N. B., R. Novak, A. Ortqvist, G. Kallenius, E. Tuomanen, and S. Normark.** 2001. Clinical isolates of *Streptococcus pneumoniae* that exhibit tolerance of vancomycin. Clin. Infect. Dis. **32:**552–558.
11. **Hidalgo, M., E. Castaneda, and C. A. Arias.** 2003. Tolerance to vancomycin in a multiresistant, Colombian isolate of *Streptococcus pneumoniae*. J. Antimicrob. Chemother. **52:**300–302.
12. **Kuroda, M., H. Kuroda, T. Oshima, F. Takeuchi, H. Mori, and K. Hiramatsu.** 2003. Two-component system VraSR positively modulates the regulation of cell-wall biosynthesis pathway in *Staphylococcus aureus*. Mol. Microbiol. **49:**807–821.
13. **Lange, R., C. Wagner, A. de Saizieu, N. Flint, J. Molnos, M. Stieger, P. Caspers, M. Kamber, W. Keck, and K. E. Amrein.** 1999. Domain organization and molecular characterization of 13 two-component systems identified by genome sequencing of *Streptococcus pneumoniae*. Gene **237:**223–234.
14. **Marchler-Bauer, A., and S. H. Bryant.** 2004. CD-Search: protein domain annotations on the fly. Nucleic Acids Res. **32:**W327–W331.
15. **Mascher, T., D. Zahner, M. Merai, N. Balmelle, A. B. De Saizieu, and R. Hakenbeck.** 2003. The *Streptococcus pneumoniae* cia regulon: CiaR target sites and transcription profile analysis. J. Bacteriol. **185:**60–70.
16. **Mascher, T., S. L. Zimmer, T. A. Smith, and J. D. Helmann.** 2004. Antibiotic-inducible promoter regulated by the cell envelope stress-sensing two-component system LiaRS of *Bacillus subtilis*. Antimicrob. Agents Chemother. **48:**2888–2896.
17. **McCullers, J. A., B. K. English, and R. Novak.** 2000. Isolation and characterization of vancomycin-tolerant *Streptococcus pneumoniae* from the cerebrospinal fluid of a patient who developed recrudescent meningitis. J. Infect. Dis. **181:**369–373.
18. **Mongodin, E., J. Finan, M. W. Climo, A. Rosato, S. Gill, and G. L. Archer.** 2003. Microarray transcription analysis of clinical *Staphylococcus aureus* isolates resistant to vancomycin. J. Bacteriol. **185:**4638–4643.
19. **Sebert, M. E., L. M. Palmer, M. Rosenberg, and J. N. Weiser.** 2002. Microarray-based identification of *htrA*, a *Streptococcus pneumoniae* gene that is regulated by the CiaRH two-component system and contributes to nasopharyngeal colonization. Infect. Immun. **70:**4059–4067.
20. **Sebert, M. E., K. P. Patel, M. Plotnick, and J. N. Weiser.** 2005. Pneumococcal HtrA protease mediates inhibition of competence by the CiaRH two-component signaling system. J. Bacteriol. **187:**3969–3979.
21. **Tettelin, H., K. E. Nelson, I. T. Paulsen, J. A. Eisen, T. D. Read, S. Peterson, J. Heidelberg, R. T. DeBoy, D. H. Haft, R. J. Dodson, A. S. Durkin, M. Gwinn, J. F. Kolonay, W. C. Nelson, J. D. Peterson, L. A. Umayam, O. White, S. L. Salzberg, M. R. Lewis, D. Radune, E. Holtzapple, H. Khouri, A. M. Wolf, T. R. Utterback, C. L. Hansen, L. A. McDonald, T. V. Feldblyum, S. Angiuoli, T. Dickinson, E. K. Hickey, I. E. Holt, B. J. Loftus, F. Yang, H. O. Smith, J. C. Venter, B. A. Dougherty, D. A. Morrison, S. K. Hollingshead, and C. M. Fraser.** 2001. Complete genome sequence of a virulent isolate of *Streptococcus pneumoniae*. Science **293:**498–506.
22. **Weigel, L. M., D. B. Clewell, S. R. Gill, N. C. Clark, L. K. McDougal, S. E. Flannagan, J. F. Kolonay, J. Shetty, G. E. Killgore, and F. C. Tenover.** 2003. Genetic analysis of a high-level vancomycin-resistant isolate of *Staphylococcus aureus*. Science **302:**1569–1571.
23. **Weiner, L., J. L. Brissette, and P. Model.** 1991. Stress-induced expression of the *Escherichia coli* phage shock protein operon is dependent on sigma 54 and modulated by positive and negative feedback mechanisms. Genes Dev. **5:**1912–1923.
24. **Zahner, D., K. Kaminski, M. van der Linden, T. Mascher, M. Meral, and R. Hakenbeck.** 2002. The *ciaR*/*ciaH* regulatory network of *Streptococcus pneumoniae*. J. Mol. Microbiol. Biotechnol. **4:**211–216.