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The cop operon is required for copper homeostasis and contributes to virulence in *Streptococcus pneumoniae*

Shafeeq, Sulman; Yesilkaya, Hasan; Kloosterman, Tomas G.; Narayanan, Geetha; Wandel, Michal; Andrew, Peter W.; Kuipers, Oscar P.; Morrissey, Julie A.

Published in:
Molecular Microbiology

DOI:
[10.1111/j.1365-2958.2011.07758.x](https://doi.org/10.1111/j.1365-2958.2011.07758.x)

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2011

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Shafeeq, S., Yesilkaya, H., Kloosterman, T. G., Narayanan, G., Wandel, M., Andrew, P. W., Kuipers, O. P., & Morrissey, J. A. (2011). The cop operon is required for copper homeostasis and contributes to virulence in *Streptococcus pneumoniae*. *Molecular Microbiology*, 81(5), 1255-1270. <https://doi.org/10.1111/j.1365-2958.2011.07758.x>

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SUPPLEMENTARY MATERIAL

Table S1. Bacterial strains and plasmids used in this study.

Strain/plasmid	Description ^a	Source
<i>S. pneumoniae</i>		
D39	Serotype 2 strain, <i>cps 2</i>	
<i>PcopY-lacZ</i>	D39 $\Delta bgaA::Pspd0633-lacZ$; Tet ^R	This study
<i>PcopY-wt-lacZ</i>	D39 $\Delta bgaA::Pspd0633-C-lacZ$; Tet ^R	This study
<i>PcopY-mut-lacZ</i>	D39 $\Delta bgaA::Pspd0633-F-lacZ$; Tet ^R	This study
<i>copY-stop</i>	D39 <i>copY</i> null mutant	This study
<i>copY-stop(PcopY-lacZ)</i>	D39 <i>copY</i> ; $\Delta bgaA::PcopY-lacZ$; Tet ^R	This study
<i>copY-stop-com</i>	D39 <i>copY</i> ; $\Delta bgaA::PcopY+copY-lacZ$; Tet ^R	This study
<i>cupA-stop</i>	D39 <i>cupA</i> null mutant	This study
$\Delta copA$	D39 <i>spd0635::Spec</i> ^R	This study
$\Delta cupA copA$	D39 <i>spd0634-5::Spec</i> ^R	This study
<i>copA</i> ⁻	D39 <i>spd0635::Spec</i> ^R	This study
<i>E. coli</i>		
EC1000	Km ^R ; MC1000 derivative carrying a single copy of the pWV1 <i>repA</i> gene in <i>glgB</i>	Laboratory collection
Plasmids		
pPP2	Amp ^R Tet ^R ; promoter-less <i>lacZ</i> . For replacement of <i>bgaA</i> with promoter <i>lacZ</i> fusion. Derivative of pTP1	Halfmann <i>et al.</i> , 2007
pORI280	Erm ^R ; <i>ori</i> ⁺ <i>repA</i> ; deletion derivative of pWV01; constitutive <i>lacZ</i> expression from P32 promoter	Leenhouts <i>et al.</i> , 1991
pSS1	pORI280 <i>copY-stop</i> , containing two stop mutations after 6 base of coding sequence	This study
pSS2	pORI280 <i>cupA-stop</i> , containing two stop mutations after 21 base of coding sequence	This study
pSS3	pPP2 <i>PcopY-lacZ</i> , containing the whole promoter	This study
pSS4	pPP2 <i>PcopY-wt-lacZ</i> , containing cop motif 1 and 2	This study
pSS5	pPP2 <i>PcopY-mut-lacZ</i> containing cop motif 1 only	This study
pSS6	pPP2 <i>PcopY+copY-lacZ</i>	This study

SUPPLEMENTARY MATERIAL

Table S2. Primers used in this study.

Name	Nucleotide sequence (5' to 3')^a	Restriction site
FcopY	CGGAATTCTTACGCACCCCTAGTGGTTG	EcoRI
RcopY	CGGGATCCGACCTGCCATTCTGCATCTG	BamHI
PcopY-C	CATGGGATCCCTCCAAAATCTACATTTGTC	BamHI
PcopY-F	CATGGGATCCACACTCATCAGTATACTC	BamHI
CopY-Com	CGGGATCCGTCTCCTTTACATAACAATTAC	BamHI
Spd 0633-1 XbaI	GCTCTAGACCACTTGTAGCAACCATAAC	XbaI
Spd 0633-2 NcoI	CCGCCATGGTTATTACTGCATTACCATACCTCC	NcoI
Spd 0633-3 NcoI	CGCCCATGGTAATAAATTTTCAGATGCAGAATGGC	NcoI
Spd 0633-4 EcoRI	GCTGGAATTCCTTTTGAAGCTGG	EcoRI
Spd-0634-1-BglII	GAAGATCTGCAATGGTCAAAGCAACGG	BglII
Spd-0634-2-NcoI	CATCCATGGTTATTAGGTTACAATACTATTTAACATGAC	NcoI
Spd-0634-3-NcoI	CATCCATGGTAATAAATTTTGTATTGCCCTTATCG	NcoI
Spd-0634-4-XbaI	TGCTCTAGAATCACAAGAGGCACAGTAAAG	XbaI
Spd 0634-1	CCTGCAATGGTCAAAGCAACGG	-
Spd 0634-2	GCATGGCGCGCCGACGTCTCCTTTACATAC	AscI
Spd 0635-1	GAAGATTATTTGGATGCAGG	-
Spd 0635-2	GCATGGCGCGCCAGTCTCCACCTACTCTAC	AscI
Spd 0635-3	GCATGGCGCGCCCTGTTCTGACCAGTTCCTTCTG	NotI
Spd 0635-4	GGGCGTTCAGCATTGTTCAAG	-
Spec-F	GCATAGGCGCGCCCTAATCAAAAATAGTGAGGAGGATAT	AscI
Spec-R	CGATTGCGGCCGCACTAAACGAAATAAACGCTAAAACG	NotI
RT-PCR primers		
Spd0633F	ACACTTTTGTCTCGGCTGGT	
Spd063R	TCCTACGGGAACAAACCTTG	
Spd0635F	TTCGTTCTTTTGGGAGCAGT	
Spd0635R	TCAGGTCGCCTACCTTGACT	
Spd0709F	TCGTGTGGCTGCCAAGCGTG	
Spd0709R	GGCTGATCCACCAGCTGAGTC	
Spd1436F	GGTCTTGGTGCAGGAGATGT	
Spd1436R	GCTGGCAATAGCCTCTTCAC	
Spd1920F	TAATGCCATCGTCAAGCAAG	
Spd1920R	TGCTCATCCTGCATCAAGAC	
Spd1118F	TACCCACCTGGTCAATAA	
Spd1118R	TCCTAACTCGTGCTGGTGTG	
MP127	CCGGGGACTTATCAGCCAACC	
MP128	TACTAGCGACGCCATCTATGTG	
CopY-F	GGAAGCTGTGATTTTCAGAG	
CupA-R	GTATCCGTTTTTTTGCTGGG	
CupA-F	GAACAGGCTGGAGAGTTTAG	
CopA-R	CCTTTTGAAGCTGGATATGGG	

^arestriction enzyme sites underlined