

**Supporting Information**

A cytosolic copper storage protein provides a second level of copper tolerance in  
*Streptomyces lividans*

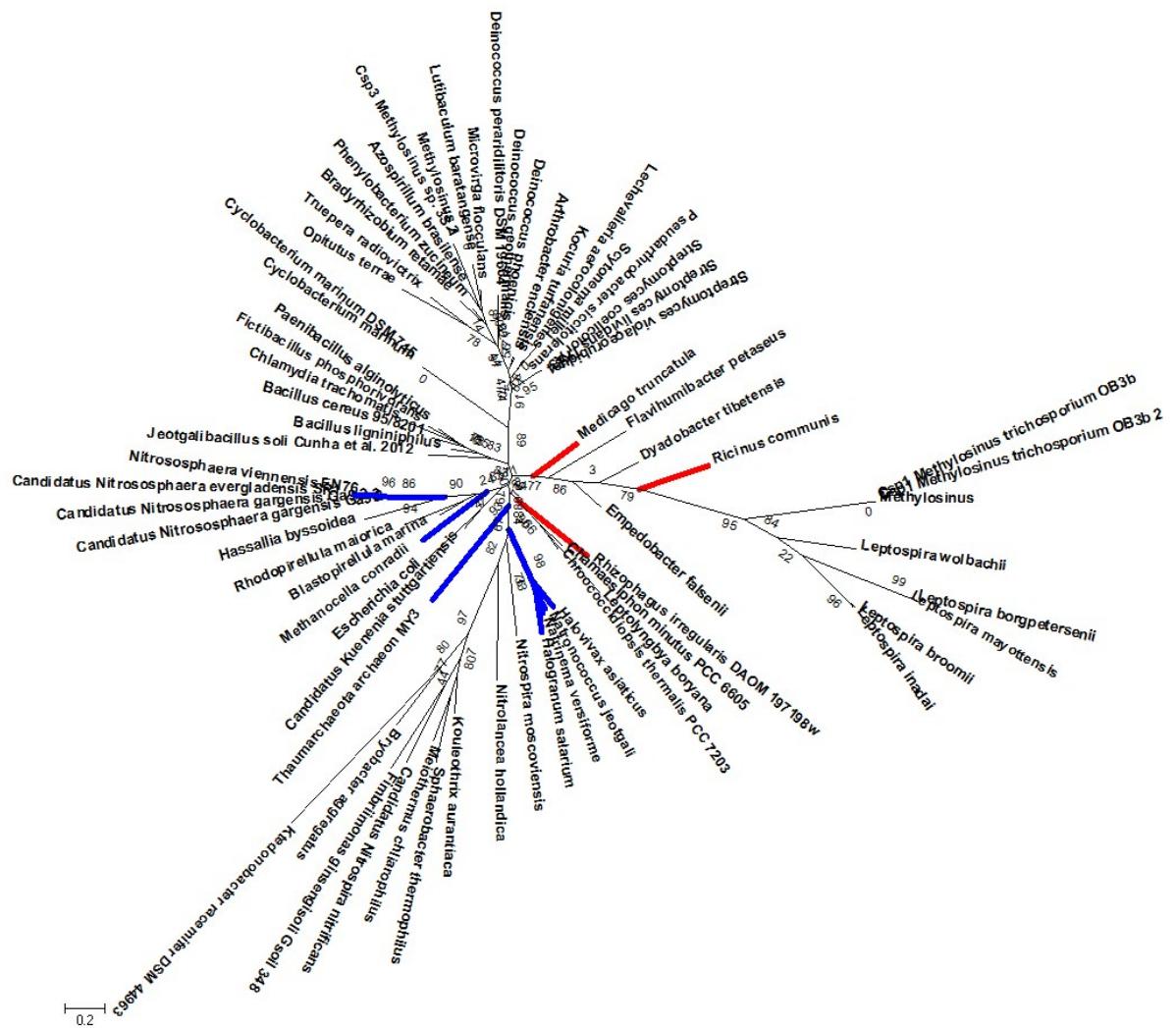
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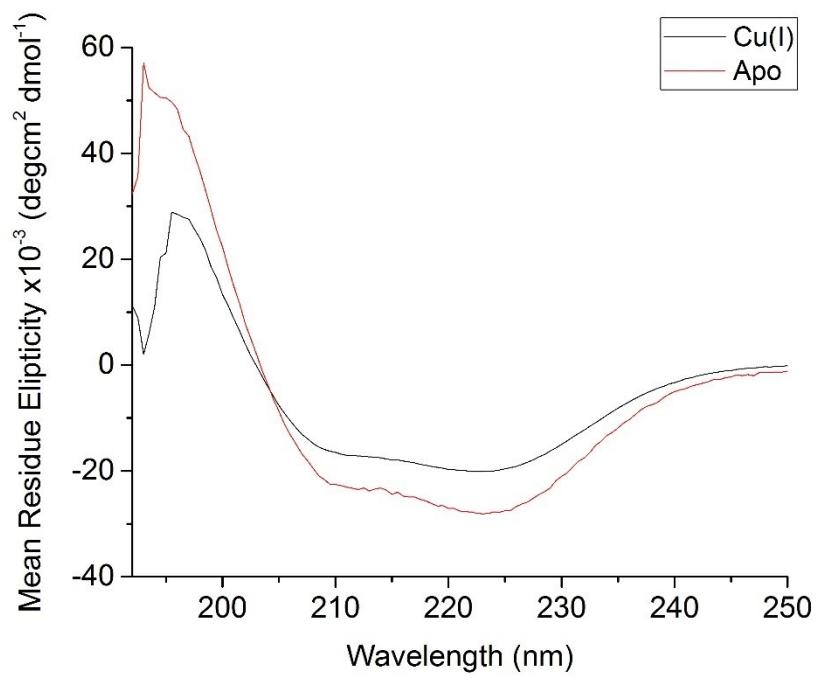
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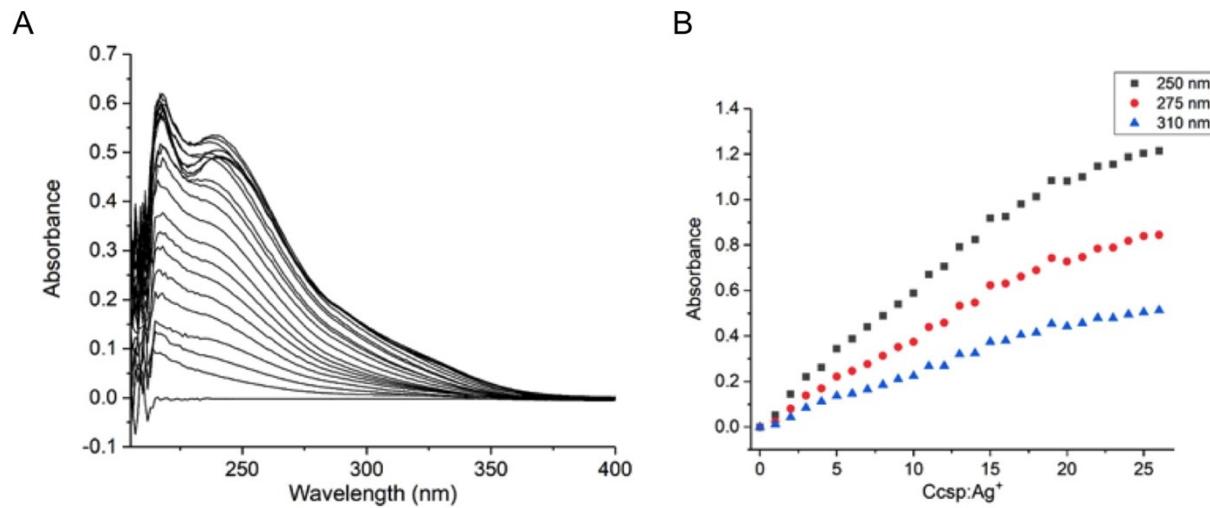
## RESULTS



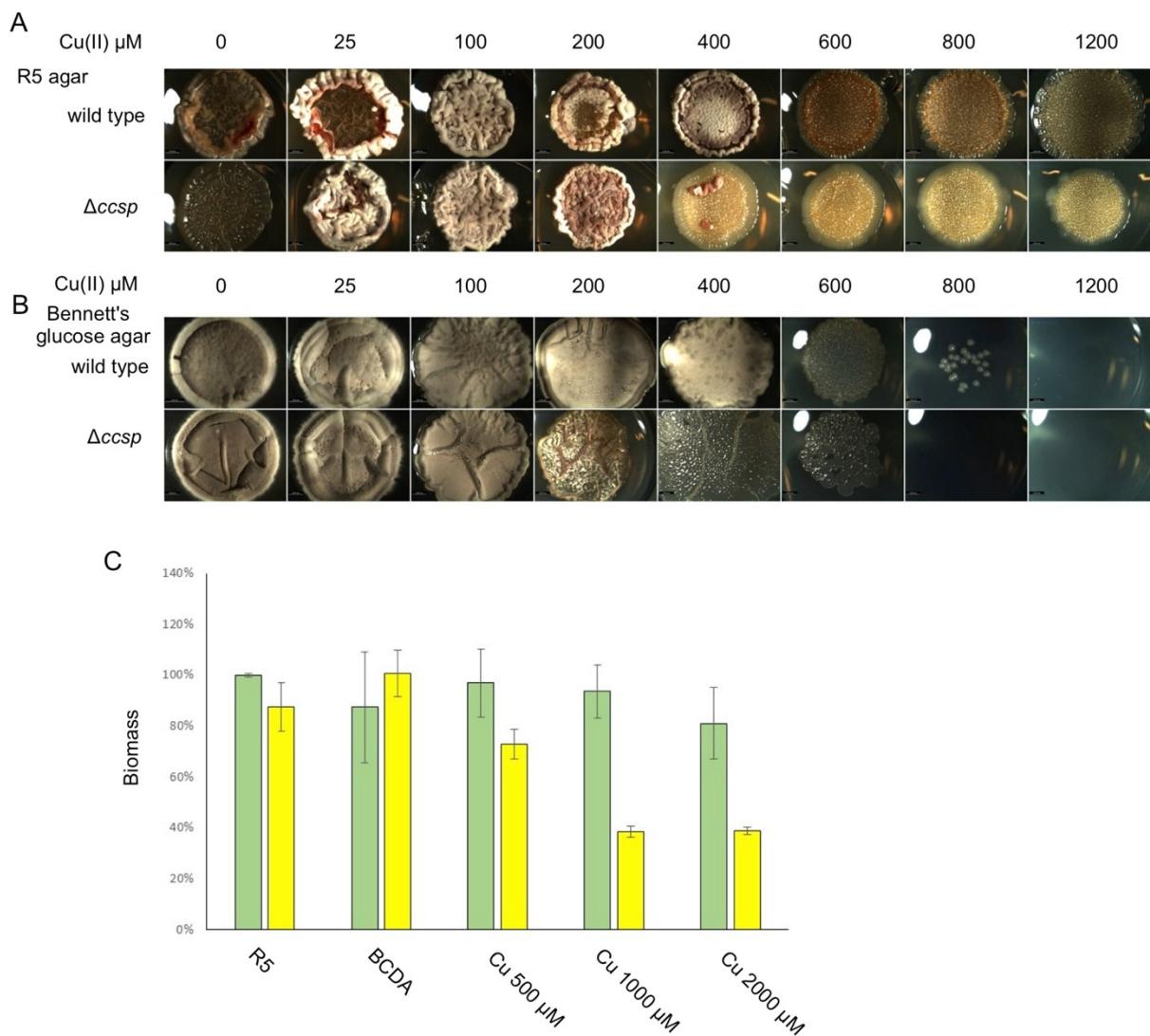
**Figure S1:** Phylogenetic tree of the *ccsp* gene. Domain Bacteria branches in black, Archaea in blue, Eukaryota in red. Node values indicate local statistical support calculated by SH test.



**Figure S2:** Far UV-CD spectra of Apo- ( $3 \mu\text{M}$ ) and  $\text{Cu}^+$ -loaded Ccsp ( $2 \mu\text{M}$ ) at pH 7.5 and 20  $^\circ\text{C}$ .



**Fig S3:**  $\text{Ag}^+$  ion binding to CcsP. A) UV-vis difference spectrum upon titration of  $\text{Ag}^+$  ions to CcsP (4  $\mu\text{M}$ ) showing the appearance of (Cys)S $\gamma$  $\rightarrow\text{Ag}^+$  LMCT bands. B) Plots of absorbance at selected wavelengths taken from (A) versus the  $\text{Ag}^+:\text{CcsP}$  concentration ratio.



**Figure S4:** The effect of Ccsp on the growth and development of *S. lividans* at 30 °C. Cu tolerance after 6 days growth of the wild type parent strain and the  $\Delta ccsp$  mutant strain on R5 agar media A) and Bennett's glucose media B). Cu(II) concentrations as indicated. All images are the same magnification with a scale bar of 2 mm. C) Biomass production after 32 h in liquid R5 cultures for the wild type (green) and the  $\Delta ccsp$  mutant strain (yellow) in the presence of the Cu chelator BCDA and various concentrations of Cu(II) citrate. The dry weight biomass of the wild type strain was set at 100 %.

**Table S1:** Taxonomic distribution of the ccsp gene in the Tree of Life.

	ccsp
<b>BACTERIA</b>	
Acidobacteria	1
Aquificae	
Caldisera	
Chrysiogenetes	
Deferribacteres	
Dictyoglomi	
Elusimicrobia	
FCB group	5
Fusobacteria	
Nitrospinae/Tectomicrobia group	
Nitrospirae	4
Proteobacteria (purple bacteria)	5
PVC group	5
Rhodothermaeota	
Spirochaetes	5
Synergistetes	
Terrabacteria group	5
Thermodesulfobacteria	
Thermotogae	
<b>Terrabacteria group</b>	
Actinobacteria	5
Armatimonadetes	1
Chloroflexi (green non-sulfur bacteria)	4
Cyanobacteria/Melainabacteria group	5
Deinococcus-Thermus	5
Firmicutes (Gram-positive bacteria)	5
Tenericutes	
<b>ARCHAEA</b>	
DPANN group	
Euryarchaeota	5
TACK group	5
<b>EUKARYOTA</b>	
Alveolata (alveolates)	
Amoebozoa	
Apusozoa	
Breviatea	
Centroheliozoa (centrohelids)	
Cryptophyta (cryptomonads)	
Euglenozoa	
Fornicata	

Glaucocystophyceae (glaucocystophytes)	
Haptophyceae (coccolithophorids)	
Heterolobosea	
Jakobida	
Katablepharidophyta	
Malawimonadidae	
Opisthokonta	1
Oxymonadida (oxymonads)	
Parabasalia (parabasalids)	
Rhizaria	
Rhodophyta (red algae)	
Stramenopiles (heterokonts)	
<u>Viriplantae (green plants)</u>	2