

Copper induces pleomorphic structures in *Sinorhizobium meliloti* 1021 altering *ctrA* gene expression

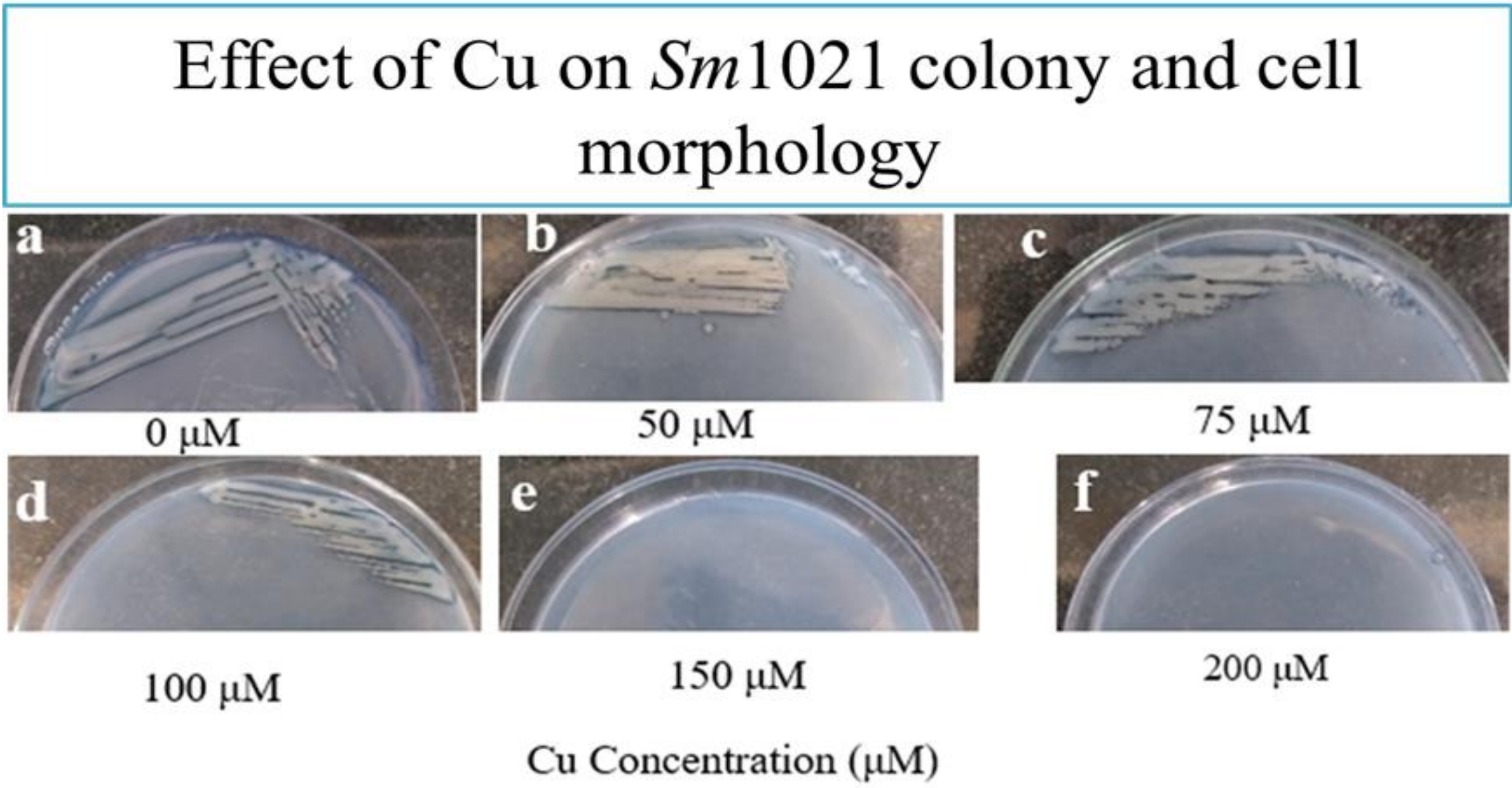
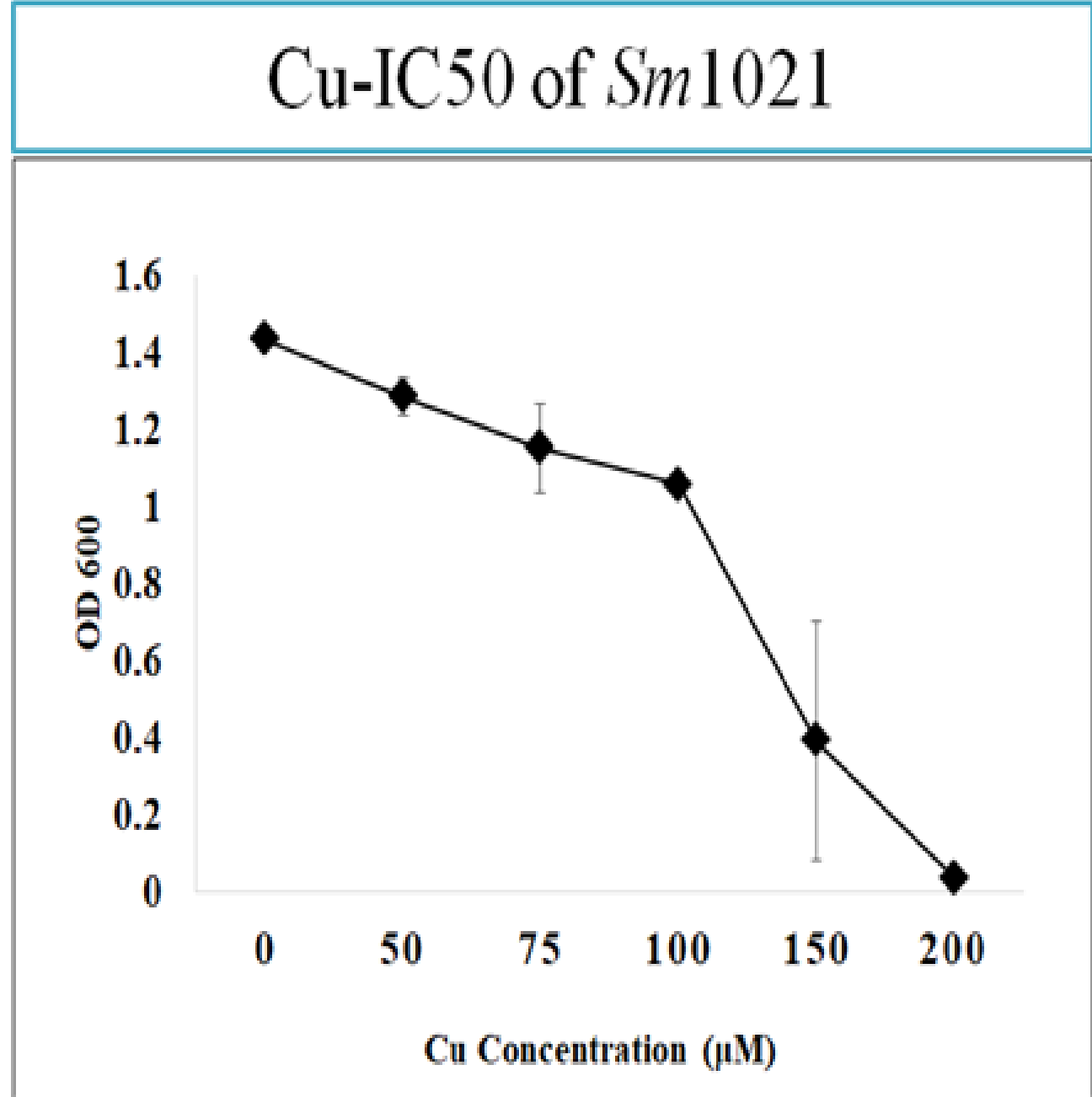
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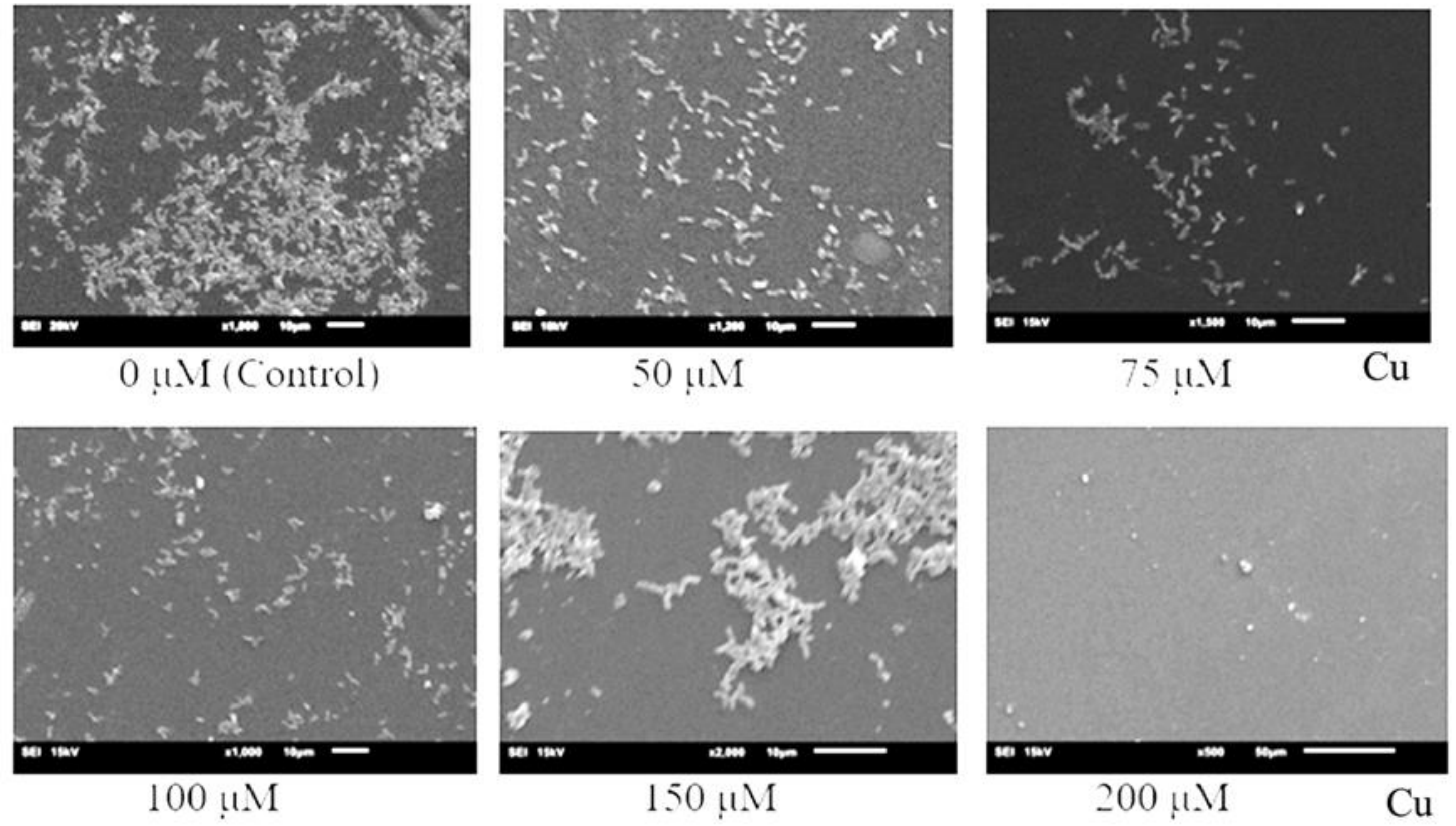


Background

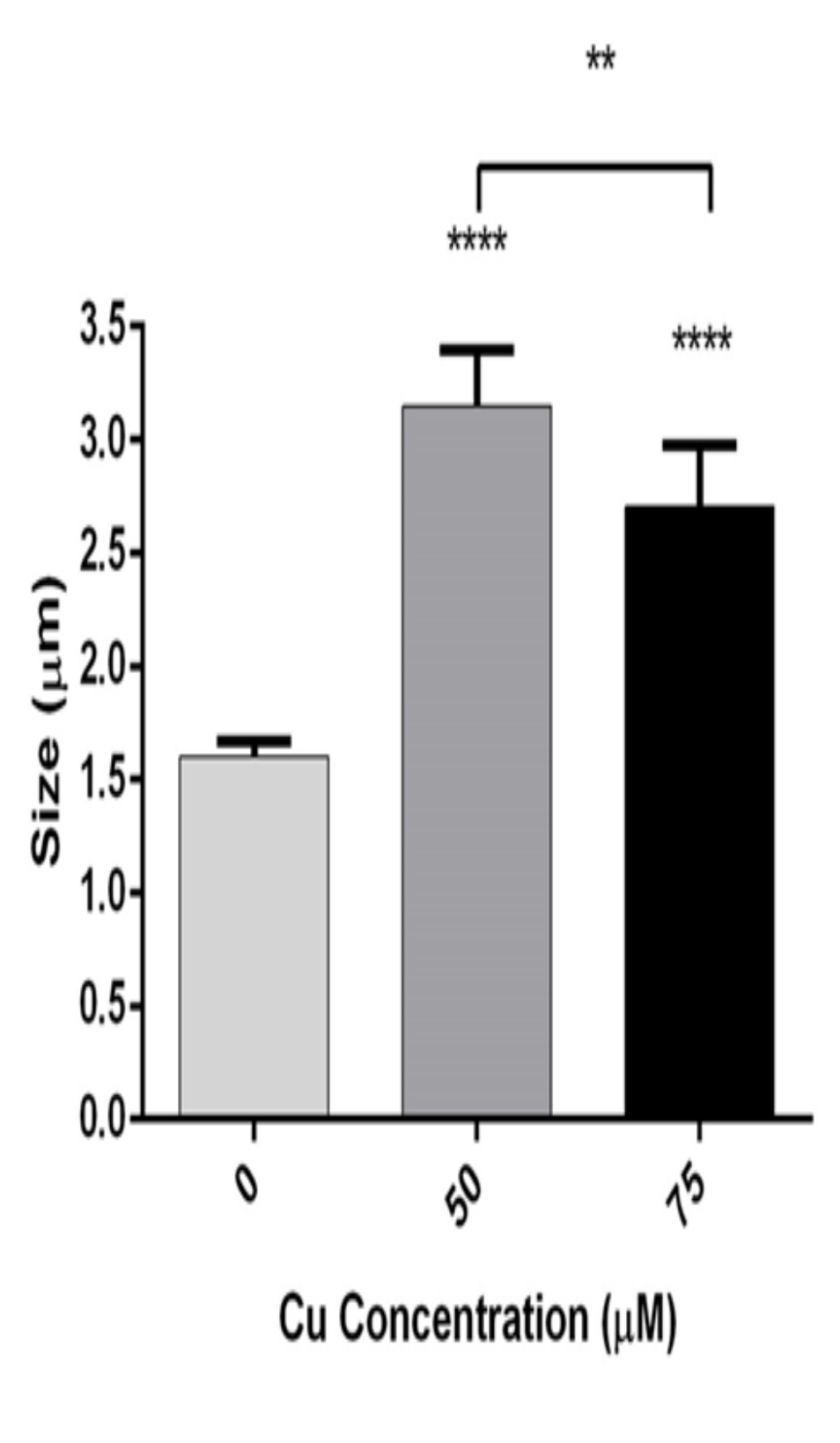
Sinorhizobium meliloti 1021 (sm1021) is a gram-negative bacterium, normally found in soil and infects the *Medicago* species to form symbiotic rhizobium legume interaction for fixing atmospheric nitrogen. During these symbiotic interactions several signalling genes and transcriptional regulators were orchestrated for successful rhizobium invasion and nodule formation. Copper (Cu) is an essential micronutrient for plant growth and acts as cofactor for microbial enzymes at optimum concentration but it becomes toxic to when it exceeds its limit. Cu is an oxidizing agent which causes oxidative damage to the cell by the production of reactive oxygen species. Copper is known to inhibit the growth of soil microflora affecting the crop yield, due to its exceeding concentration by urbanisation and industrialisation. Our earlier studies have shown that Cu affects the growth of *Medicago truncatula* and *S. meliloti* growth. Furthur we extended our research to understand how Cu is affecting the *S.meliloti* morphology by altering gene functions or else the metal is inducing functional bacteroid like structures.



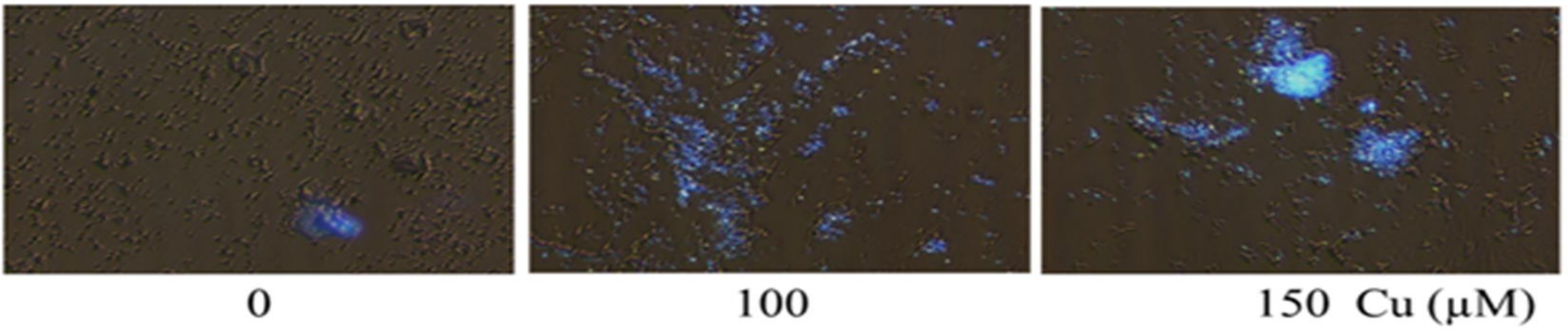
Electron microscopy of *Sm*1021 cells under Cu stress



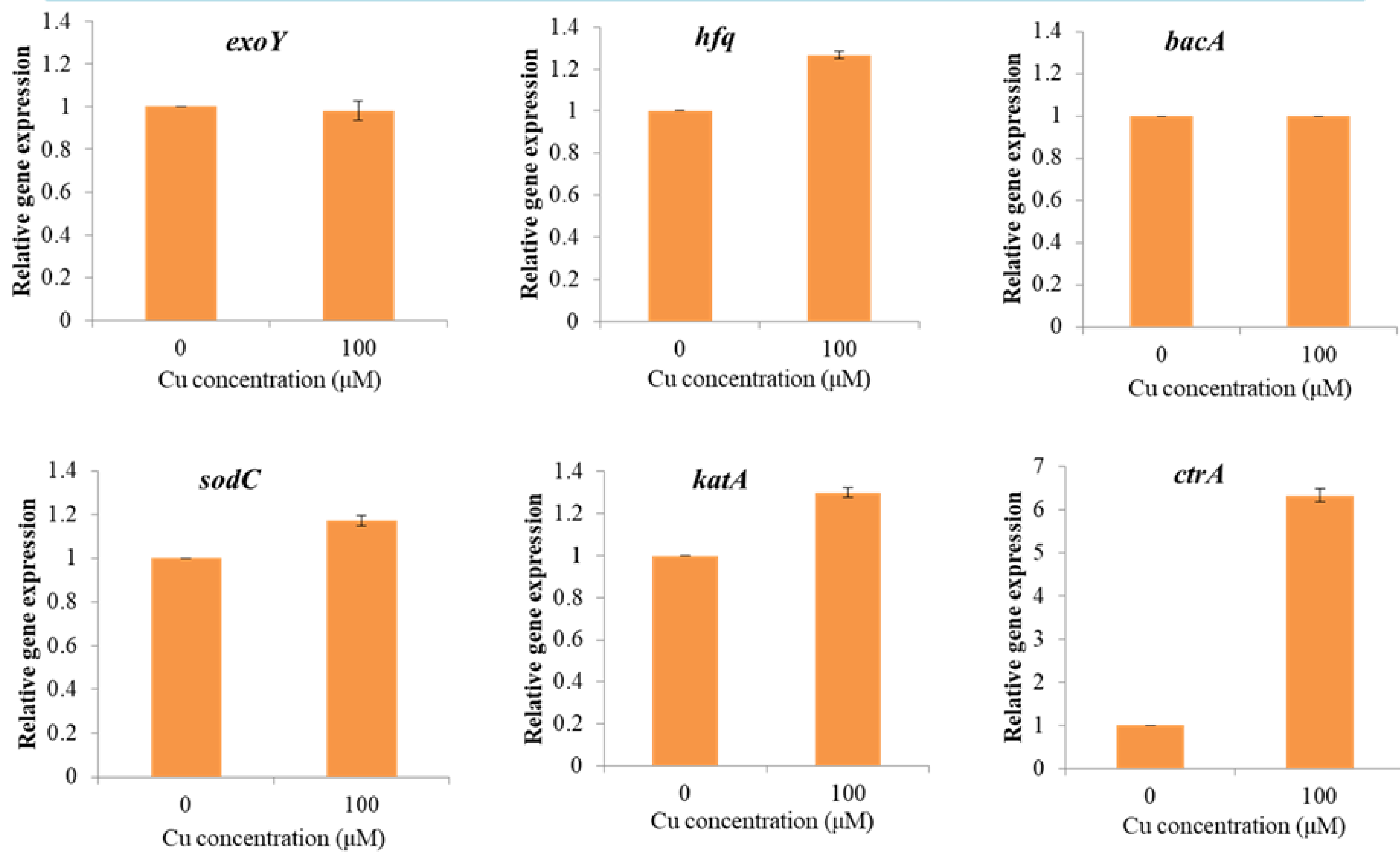
Effect of Cu on cell size of *Sm*1021



Fluorescence Microscopy of *Sm*1021 cells (DAPI staining)



Gene Expression Analysis of *Sm*1021 cells under Cu stress



Summary

- The Cu- IC50 value of *Sm*1021 clearly shows that 50% growth was inhibited at 100µM Cu concentration along with cell envelope damage as evident from Sudan black staining.
- At 50 µM Cu, cells were elongated and at further higher Cu concentration undifferentiated bacteroid like structure are formed with increased nuclear content. The oxidative stress responsive genes and cell cycle gene regulator were up regulated with no change in *BacA* gene expression.
- Root Attachment Assay (data not shown) infers that Cu treated *Sm*1021 cells were unable to attach the roots of *Medicago* seedlings in comparison to untreated cells.

Conclusion

Cu induced certain morphological changes in *S. meliloti* 1021 exhibiting pleomorphism mimicking to the bacteroid like structure present in legume root nodules. In *S.meliloti* 1021, although bacteroid like structures are formed they were found to be non-functional (no change in *BacA* expression). In fact Cu stress has altered the master cell cycle regulator *ctrA* to induce pleomorphism. Additionally oxidative stress responsive genes (*sodC*, *kat A*) and RNA chaperon *hfq* were induced positively in *S. meliloti* 1021cells to confer resistance against Cu stress.

Reference
Murali Sharaff and G Archana, (2016). Copper-induced modifications in early symbiotic signaling factors of *Ensifer (Sinorhizobium)-Medicago* interactions. Archives of Microbiology 198(7):701-9.