

# Biogenic Synthesis of Copper Nanoparticles Using Bacterial Strains Isolated from an Antarctic Consortium Associated to a Psychrophilic Marine Ciliate: Characterization and Potential Application as Antimicrobial Agents

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## Supplementary figures

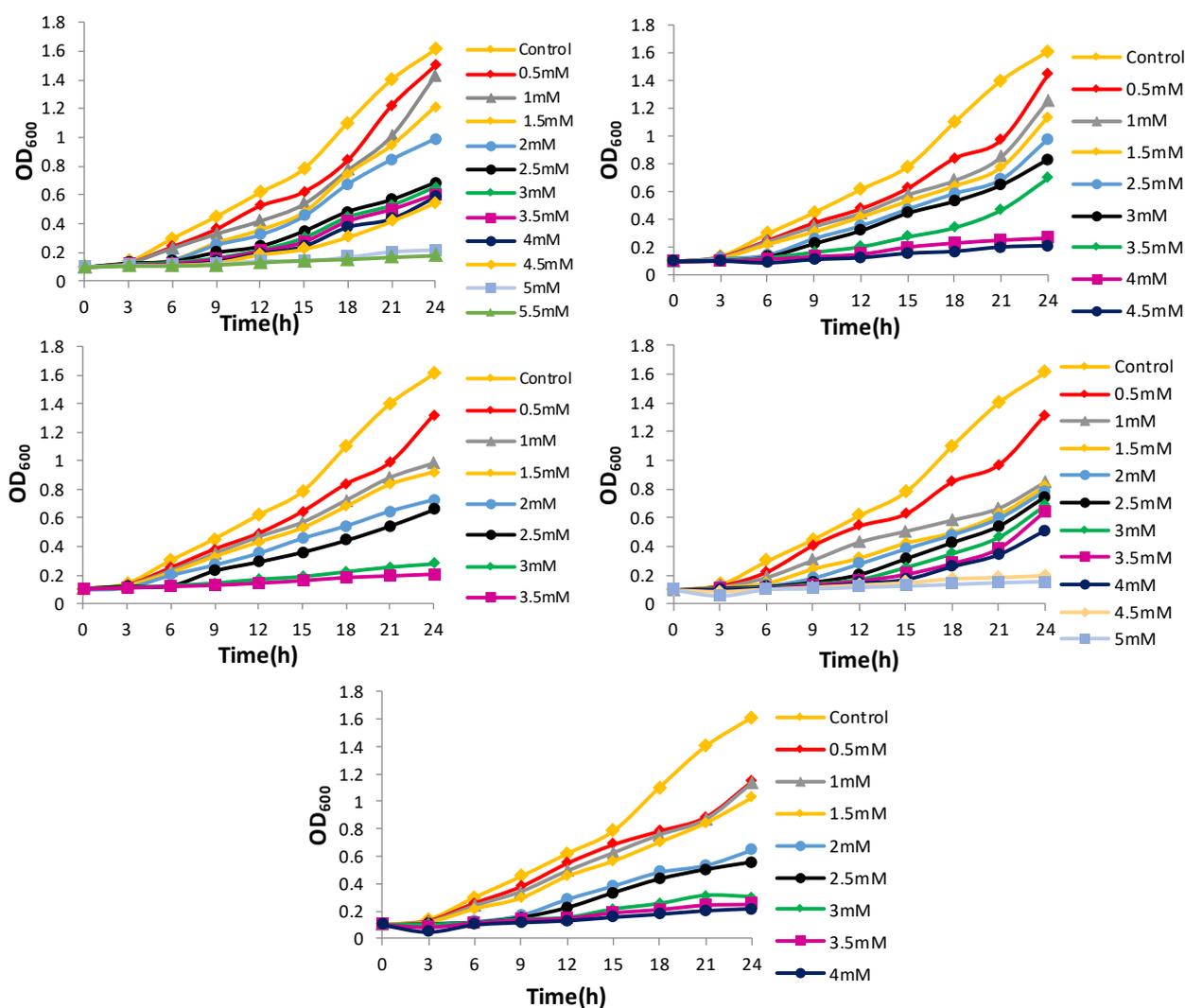
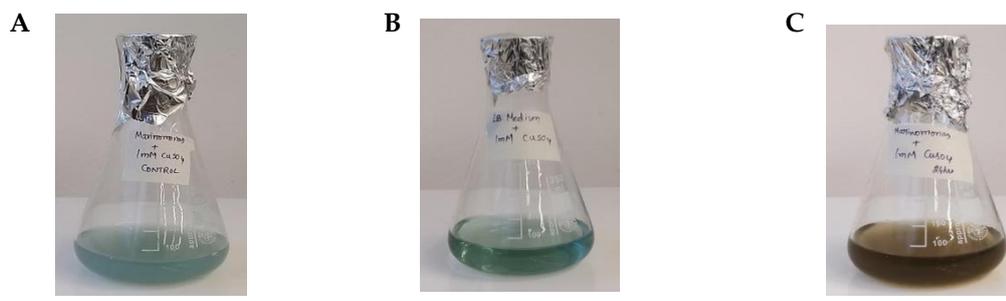
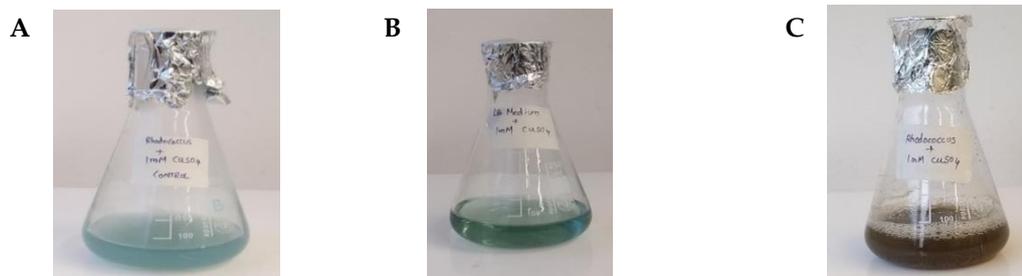


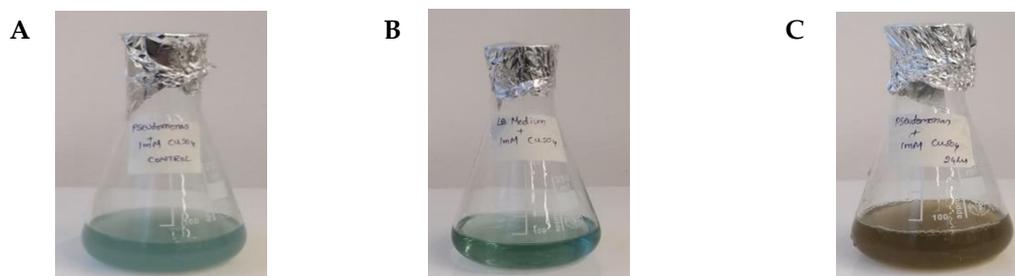
Figure S1. Bacterial growth in the presence of increasing concentration of CuSO<sub>4</sub>.



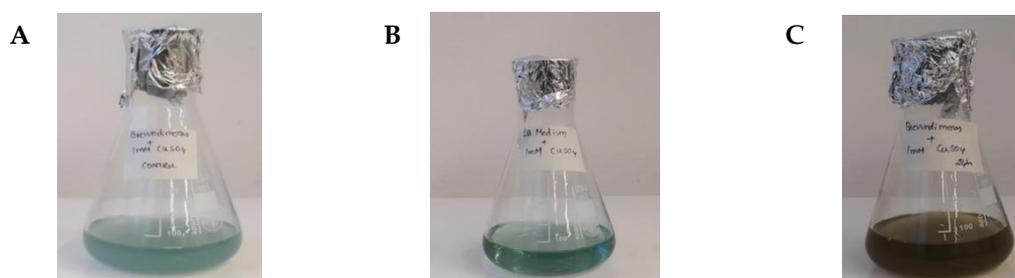
*Marinomonas ef1*



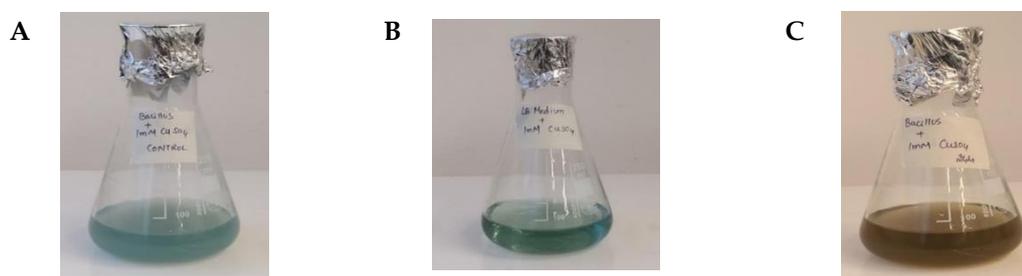
*Rhodococcus ef1*



*Pseudomonas ef1*



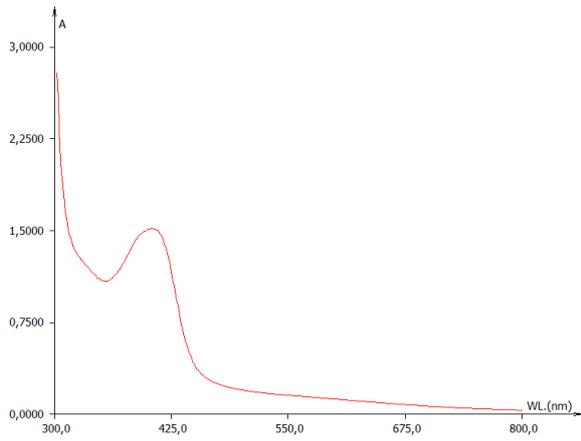
*Brevundimonas ef1*



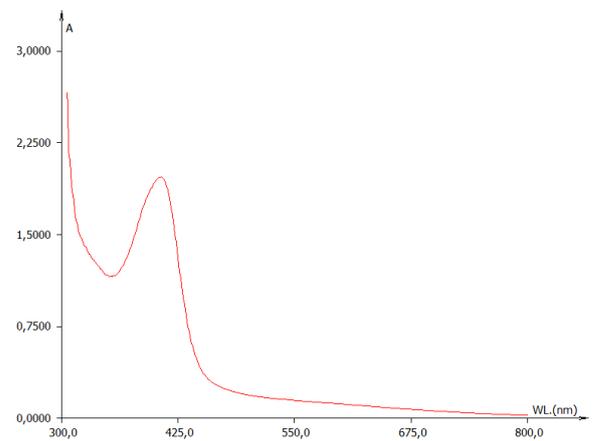
*Bacillus ef1*

**Figure. S2.** Biosynthesis of Cu NPs from *Marinomonas ef1*, *Rhodococcus ef1*, *Pseudomonas ef1*, *Brevundimonas ef1* and *Bacillus ef1*. **A.** Control with heat killed bacterial culture with 1mM CuSO<sub>4</sub>; **B.** Control with LB medium with 1mM CuSO<sub>4</sub>; **C.** Biosynthesized Cu NPs.

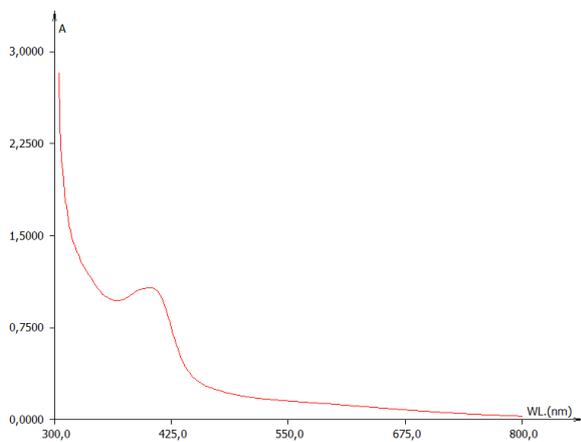
**A) *Rhodococcus* ef1**



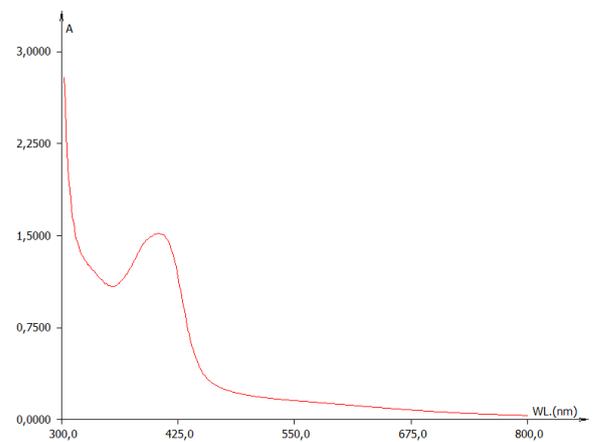
**B) *Pseudomonas* ef1**



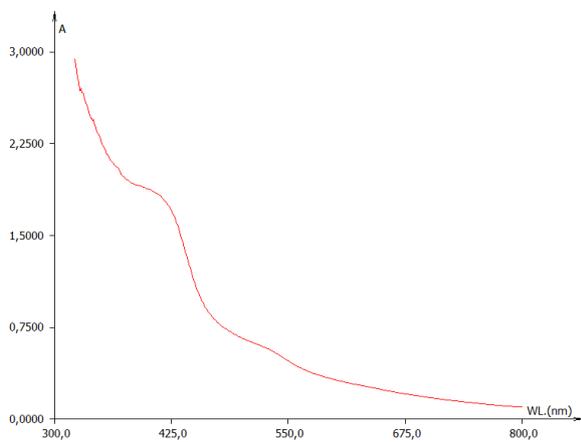
**C) *Brevundimonas* ef1**



**D) *Bacillus* ef1**

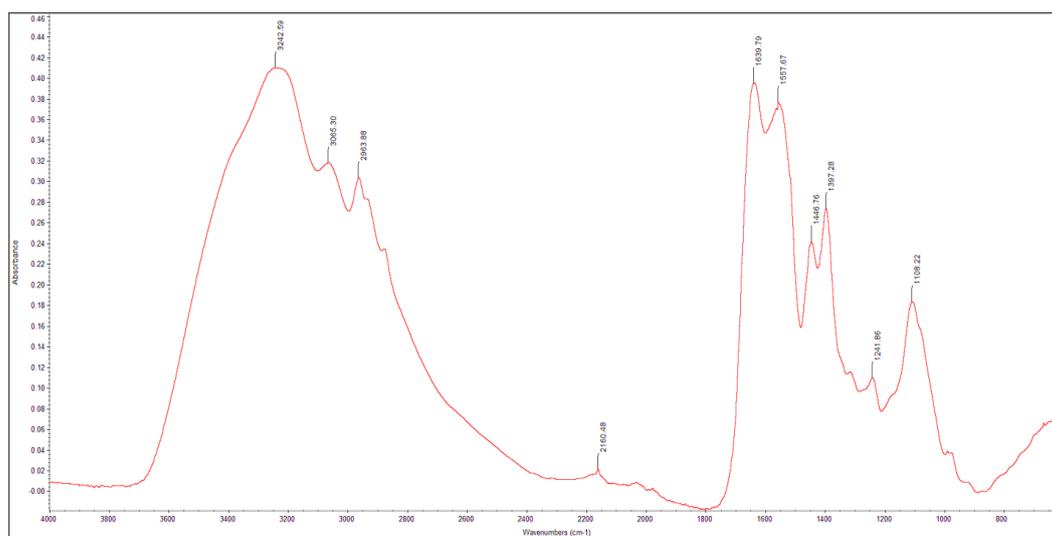


**E) *Marinomonas* ef1**



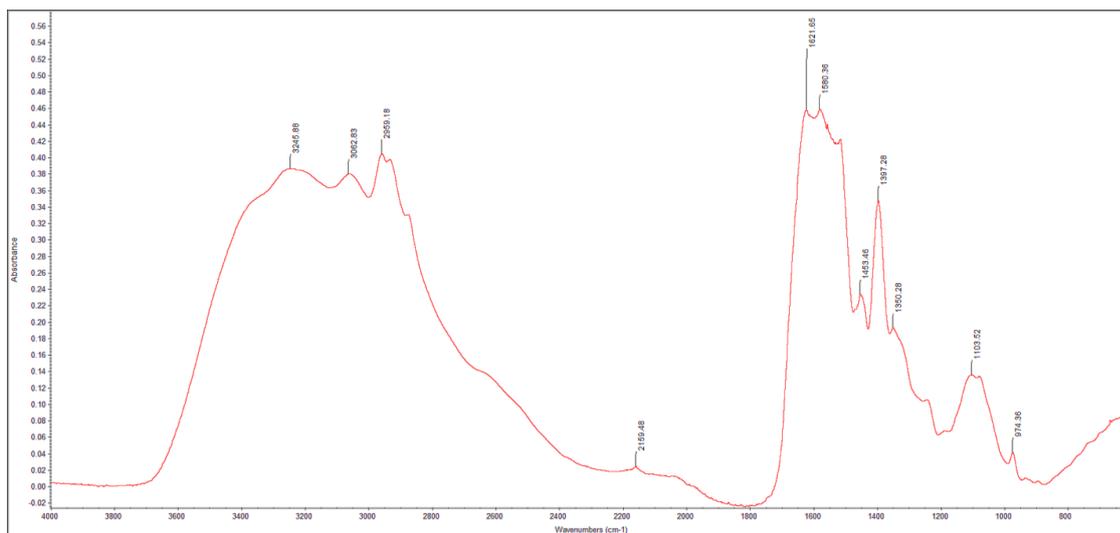
**Figure S3.** UV-Vis absorbance spectra of bio- CuO NPs synthesized from (A) *Rhodococcus* ef1, (B) *Pseudomonas* ef1, (C) *Brevundimonas* ef1; (D) *Bacillus* ef1; (E) *Marinomonas* ef1.

IR spectra. All the samples showed similar IR spectra with comparable vibration peaks. In the IR spectra a broad intense absorption band in the region between 3600 and 2800  $\text{cm}^{-1}$  can be attributed to  $-\text{OH}$  stretching, the peak at around 3245  $\text{cm}^{-1}$  can be attributed to  $-\text{NH}$  of amide. The band at around 3065  $\text{cm}^{-1}$  represents the  $=\text{C}-\text{H}$  stretch of aromatic and unsaturated hydrocarbons; on the other hand the band at around 2960  $\text{cm}^{-1}$  is due to the  $\text{C}-\text{H}$  stretching of the aliphatic portion of the molecule around nanoparticles. In the range between 2100 to 1800  $\text{cm}^{-1}$  the bands can be assigned to the aromatic portion inside the biomolecules. At around 1620–1650 it is possible to observe the carbonyl group  $\text{C}=\text{O}$  of amide and also in this region can be possible the observe the bending of the unsaturated  $\text{C}=\text{C}$  bending; in addition, the band at around 1550  $\text{cm}^{-1}$  is representative of the  $\text{N}-\text{H}$  bending of the secondary amide. The peak at around 1440–1455  $\text{cm}^{-1}$  indicates the bending of the aliphatic  $-\text{C}-\text{H}$  and  $-\text{CH}_3$ . The band at around 1390  $\text{cm}^{-1}$  can be attributed to the  $-\text{OH}$  bending. The peak at around 1240  $\text{cm}^{-1}$  can be assigned to  $\text{C}-\text{N}$  stretching vibration of amide, and the peak at around 1100  $\text{cm}^{-1}$  to the  $\text{C}-\text{O}$  stretching of alcohol. The broad adsorption at around 900–800  $\text{cm}^{-1}$  can be due to the rocking vibration out of plane of  $-\text{NH}$  portion of the amide.



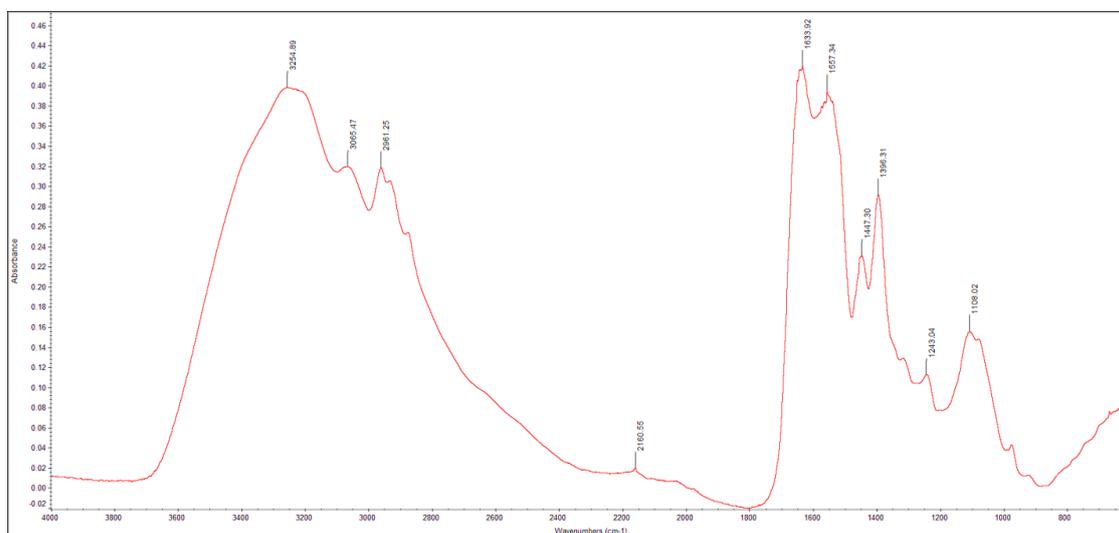
IR Absorption ( $\text{cm}^{-1}$ )	Band Assignments
3242.59	$-\text{NH}$ stretch
3065.30	$=\text{C}-\text{H}$ stretch of aromatic and unsaturated hydrocarbons
2963.88	$-\text{CH}$ stretch of aliphatic compounds
1639.79	$-\text{C}=\text{O}$ stretching amide, bending $\text{C}=\text{C}$
1557.67	$-\text{NH}$ in plane bend amide
1446.76	$-\text{C}-\text{H}$ and $-\text{CH}_3$ in Aliphatic -compounds
1397.28	$-\text{OH}$ bending
1241	$\text{C}-\text{N}$ stretch amide
1108.82	$\text{C}-\text{O}$ stretching

**Figure S4.** FTIR spectrum of biosynthesized CuO NPs from *Marinomonas ef1* and IR assignments.



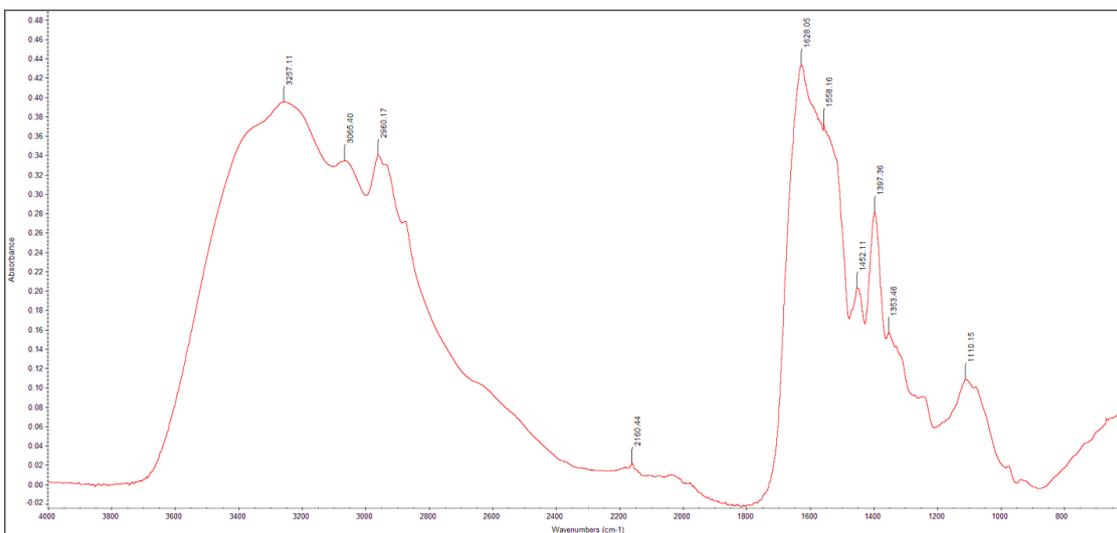
IR Absorption (cm <sup>-1</sup> )	Band Assignments
3245.88	-NH stretch
3062.83	=C-H stretch of aromatic and unsaturated hydrocarbons
2959.18	-CH stretch of aliphatic compounds
1621.65	-C=O stretching amide, bending C=C
1580.36	-NH in plane bend amide
1453.46	-C-H and -CH <sub>3</sub> in Aliphatic -compounds
1397.28	-OH bending
1240	C-N stretch amide
1103.52	C-OH stretching

Figure S5. FTIR spectrum of biosynthesized CuO NPs from *Rhodococcus* ef1 and IR assignments.



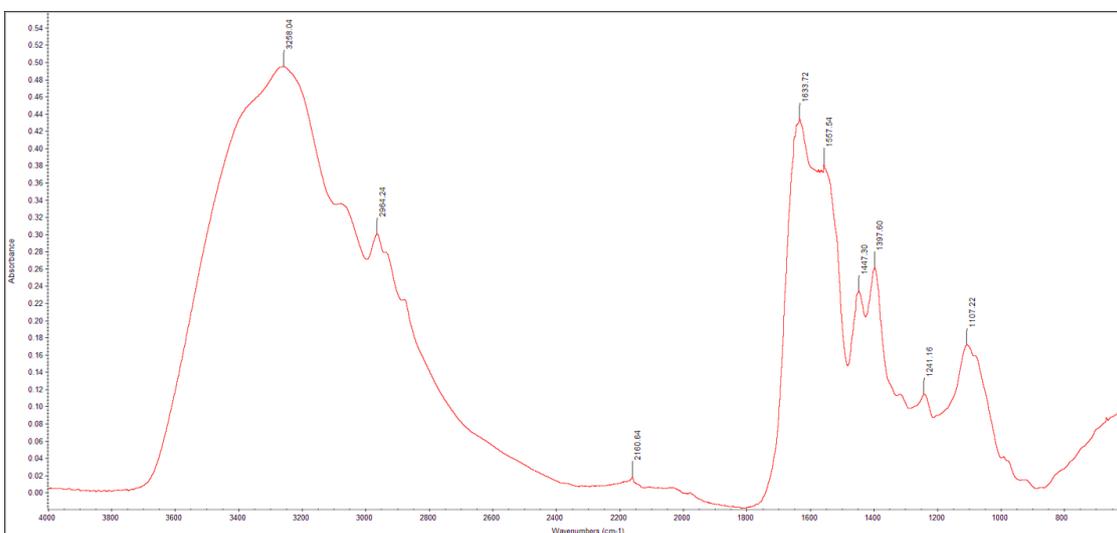
IR Absorption (cm <sup>-1</sup> )	Band Assignments
3254.89	-NH stretch
3065.47	=C-H stretch of aromatic and unsaturated hydrocarbons
2961.25	-CH stretch of aliphatic compounds
1633.92	-C=O stretching amide, bending C=C
1557.34	-NH in plane bend amide
1447.30	-C-H and -CH <sub>3</sub> in Aliphatic -compounds
1396.31	-OH bending
1243.04	C-N stretch amide
1108.02	C-OH stretching

Figure S6. FTIR spectrum of biosynthesized CuO NPs from *Pseudomonas* ef1 and IR assignments.



IR Absorption (cm <sup>-1</sup> )	Band Assignments
3257.11	-NH stretch
3065.40	=C-H stretch of aromatic and unsaturated hydrocarbons
2960.70	-CH stretch of aliphatic compounds
1628.01	-C=O stretching amide, bending C=C
1558.16	-NH in plane bend amide
1452.11	-C-H and -CH <sub>3</sub> in Aliphatic -compounds
1397.36	-OH bending
1240.21	C-N stretch amide
1110.15	C-OH stretching

Figure S7. FTIR spectrum of biosynthesized CuONPs from *Brevundimonas ef1* and IR assignments.



IR Absorption (cm <sup>-1</sup> )	Band Assignments
3258.04	-NH stretch
3064.25	=C-H stretch of aromatic and unsaturated hydrocarbons
2964.24	-CH stretch of aliphatic compounds
1633.72	-C=O stretching amide, bending C=C
1557.54	-NH in plane bend amide
1447.30	-C-H and -CH <sub>3</sub> in Aliphatic -compounds
1397.60	-OH bending
1241.16	C-OH stretching

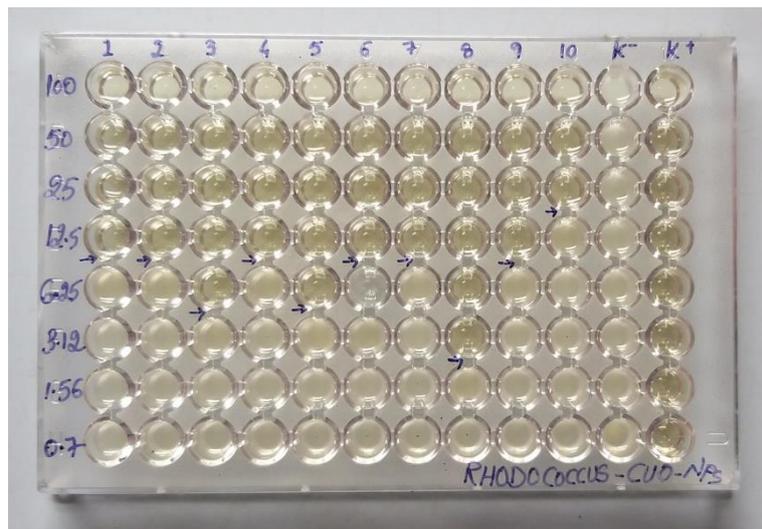
Figure S8. FTIR spectrum of biosynthesized CuO NPs from *Bacillus ef1* and IR assignments.

**Figure S9. MIC values of *Marinomonas* CuO NPs.** Among Gram-negative bacteria *Proteus mirabilis* showed the lowest MIC of 3.12 µg/mL whereas *Pseudomonas sp.*, *Citrobacter koseri* and *Serratia marcescens* showed a MIC of 6.25 µg/mL. While *Escherichia coli*, *Klebsiella pneumoniae* and *Acinetobacter baumannii* showed a MIC value of 12.5 µg/mL. In gram positive bacteria *Staphylococcus aureus* showed 12.5 µg/mL. Among fungi the lowest MIC value was in *Candida parapsilosis* (12.5 µg/mL), while *Candida albicans* showed a MIC of 25 µg/mL. The lowest MBC among gram negative bacteria was showed by *Proteus mirabilis* and *Citrobacter koseri* is 6.25 µg/mL. *Pseudomonas sp.*, *Acinetobacter baumannii* and *Serratia marcescens* showed a MBC of 12.5 µg/mL. Among gram positive bacteria *Staphylococcus aureus* showed a MBC of 25 µg/mL. Among Fungi *Candida albicans* and *Candida parapsilosis* showed MBC value of 25 µg/mL.



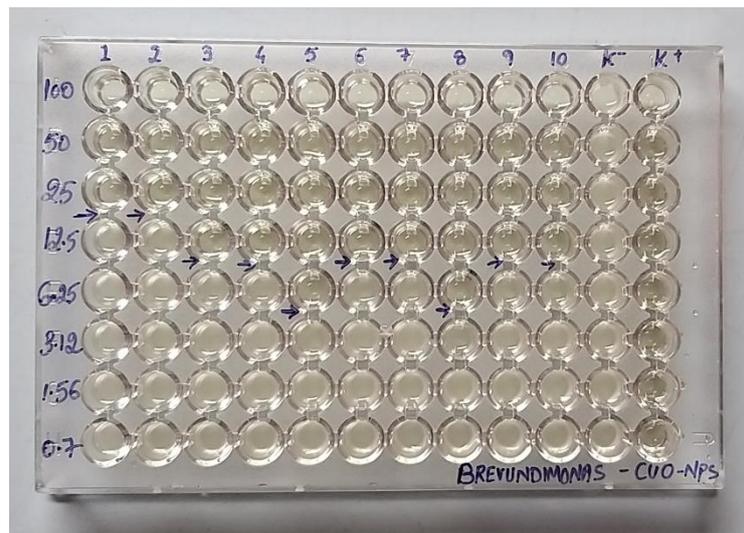
*Marinomonas* ef1 CuO NPs Broth microdilution method. 1. *Staphylococcus aureus* 2. *Escherichia coli* 3. *Klebsiella pneumoniae* 4. *Pseudomonas sp.* 5. *Proteus mirabilis* 6. *Citrobacter koseri* 7. *Acinetobacter baumannii* 8. *Serratia marcescens* 9. *Candida albicans* 10. *Candida parapsilosis*.

**Figure S10. MIC values of *Rhodococcus ef1* CuO NPs.** Among Gram-negative bacteria *Serratia marcescens* showed the lowest MIC (3.12  $\mu\text{g/mL}$ ) whereas *Klebsiella pneumoniae* and *Proteus mirabilis* showed a MIC of 6.25  $\mu\text{g/mL}$ . *Escherichia coli*, *Pseudomonas sp.*, *Citrobacter koseri* and *Acinetobacter baumannii* showed a MIC value of 12.5  $\mu\text{g/mL}$ . In gram-positive bacteria, *Staphylococcus aureus* showed a MIC of 12.5  $\mu\text{g/mL}$ . The lowest MIC in fungi was in *Candida albicans* (12.5  $\mu\text{g/mL}$ ). *Candida parapsilosis* showed a MIC of 25  $\mu\text{g/mL}$ . The lowest MBC among gram-negative bacteria was showed by *Klebsiella pneumoniae*, *Proteus mirabilis*, *Citrobacter koseri*, *Acinetobacter baumannii* and *Serratia marcescens* (12.5  $\mu\text{g/mL}$ ). *Escherichia coli* and *Pseudomonas sp.* showed a MBC of 25  $\mu\text{g/mL}$ . Among gram-positive bacteria *Staphylococcus aureus* showed a MBC of 25  $\mu\text{g/mL}$ . *Candida albicans* and *Candida parapsilosis* also showed a MBC of 25  $\mu\text{g/mL}$ .



*Rhodococcus ef1* CuO NPs Broth microdilution method. 1. *Staphylococcus aureus* 2. *Escherichia coli* 3. *Klebsiella pneumoniae* 4. *Pseudomonas sp.* 5. *Proteus mirabilis* 6. *Citrobacter koseri* 7. *Acinetobacter baumannii* 8. *Serratia marcescens* 9. *Candida albicans* 10. *Candida parapsilosis*.

**Figure S11. MIC values of *Brevundimonas ef1* CuO NPs.** *Proteus mirabilis* and *Serratia marcescens* showed the lowest MIC (6.25 µg/mL), whereas *Klebsiella pneumoniae*, *Pseudomonas sp.*, *Citrobacter koseri* and *Acinetobacter baumannii* showed a MIC value of 12.5 µg/mL. *Escherichia coli* showed a MIC value of 25 µg/mL. In gram-positive bacteria, *Staphylococcus aureus* showed a MIC value of 25 µg/mL. Among fungi, *Candida albicans* and *Candida parapsilosis* showed a MIC value of 12.5 µg/mL. The lowest MBC value among gram negative bacteria was by *Proteus mirabilis* and *Serratia marcescens* (12.5 µg/mL). *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas sp.*, *Citrobacter koseri* and *Acinetobacter baumannii* showed a MBC value of 25 µg/mL. Among gram positive bacteria *Staphylococcus aureus* showed a MBC of 25 µg/mL. *Candida albicans* and *Candida parapsilosis* showed a MBC value of 25 µg/mL.



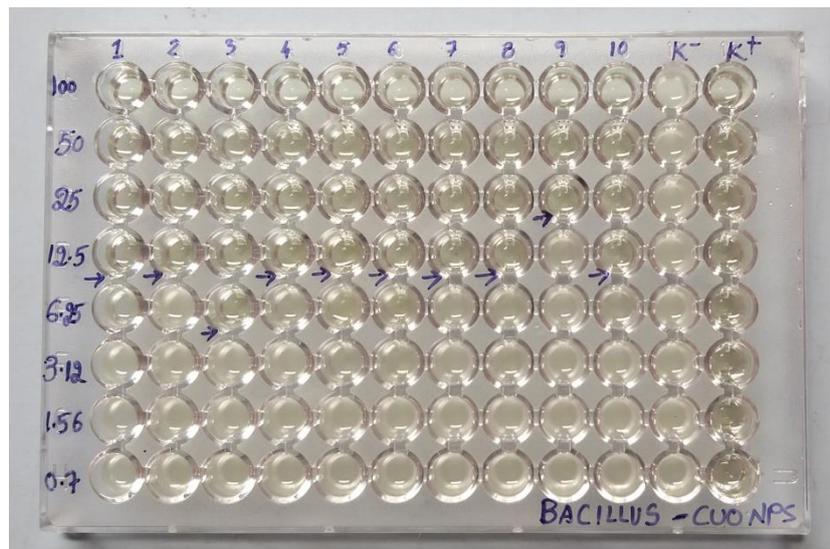
*Brevundimonas ef1* CuO NPs Broth microdilution method. 1. *Staphylococcus aureus* 2. *Escherichia coli* 3. *Klebsiella pneumoniae* 4. *Pseudomonas sp.* 5. *Proteus mirabilis* 6. *Citrobacter koseri* 7. *Acinetobacter baumannii* 8. *Serratia marcescens* 9. *Candida albicans* 10. *Candida parapsilosis*.

**Figure S12. MIC values of *Pseudomonas* ef1 CuO NPs.** *Proteus mirabilis*, *Citrobacter koseri* and *Serratia marcescens* showed the lowest MIC (6.25 µg/mL). *Klebsiella pneumoniae*, *Pseudomonas sp.*, and *Acinetobacter baumannii* showed a MIC value of 12.5 µg/mL. *Escherichia coli* showed a MIC value of 25 µg/mL. In gram positive bacteria *Staphylococcus aureus* showed a MIC value of 12.5 µg/mL. Among fungi *Candida albicans* and *Candida parapsilosis* showed MIC value of 25 µg/mL and 12.5 µg/mL respectively. The Lowest MBC was in *Pseudomonas sp.*, *Proteus mirabilis*, *Citrobacter koseri*, *Acinetobacter baumannii* and *Serratia marcescens* (12.5 µg/mL). *Escherichia coli* and *Klebsiella pneumoniae* showed a MBC value of 25 µg/mL. Among gram positive bacteria *Staphylococcus aureus* showed a MBC of 25 µg/mL. *Candida albicans* and *Candida parapsilosis* showed a MBC value of 25 µg/mL.



*Pseudomonas* ef1 CuO NPs Broth microdilution method. 1. *Staphylococcus aureus* 2. *Escherichia coli* 3. *Klebsiella pneumoniae* 4. *Pseudomonas sp.* 5. *Proteus mirabilis* 6. *Citrobacter koseri* 7. *Acinetobacter baumannii* 8. *Serratia marcescens* 9. *Candida albicans* 10. *Candida parapsilosis*.

**Figure S13. MIC values of *Bacillus ef1* CuO NPs.** *Klebsiella pneumoniae*, *Proteus mirabilis* and *Serratia marcescens* showed the lowest MIC (6.25  $\mu\text{g/mL}$ ), whereas *Escherichia coli*, *Pseudomonas sp.*, *Citrobacter koseri* and *Acinetobacter baumannii* showed a MIC of 12.5  $\mu\text{g/mL}$ . *Escherichia coli* showed a MIC of 25  $\mu\text{g/mL}$ . In gram positive bacteria *Staphylococcus aureus* showed a MIC value of 12.5  $\mu\text{g/mL}$ . Among fungi *Candida albicans* and *Candida parapsilosis* showed a MIC of 12.5  $\mu\text{g/mL}$  and 6.25  $\mu\text{g/mL}$  respectively. The lowest MBC value among gram-negative bacteria was in *Klebsiella pneumoniae* (6.25  $\mu\text{g/mL}$ ). *Escherichia coli*, *Pseudomonas sp.*, *Proteus mirabilis*, *Citrobacter koseri*, *Acinetobacter baumannii* and *Serratia marcescens* showed a MBC of 12.5  $\mu\text{g/mL}$ . Among gram positive bacteria *Staphylococcus aureus* showed a MBC of 12.5  $\mu\text{g/mL}$ . Among Fungi *Candida albicans* and *Candida parapsilosis* showed MBC of 25  $\mu\text{g/mL}$  and 12.5  $\mu\text{g/mL}$  respectively.



*Bacillus ef1* CuO NPs Broth microdilution method .1. *Staphylococcus aureus* 2. *Escherichia coli* 3. *Klebsiella pneumoniae* 4. *Pseudomonas sp.* 5. *Proteus mirabilis* 6. *Citrobacter koseri* 7. *Acinetobacter baumannii* 8. *Serratia marcescens* 9. *Candida albicans* 10. *Candida parapsilosis*.