

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

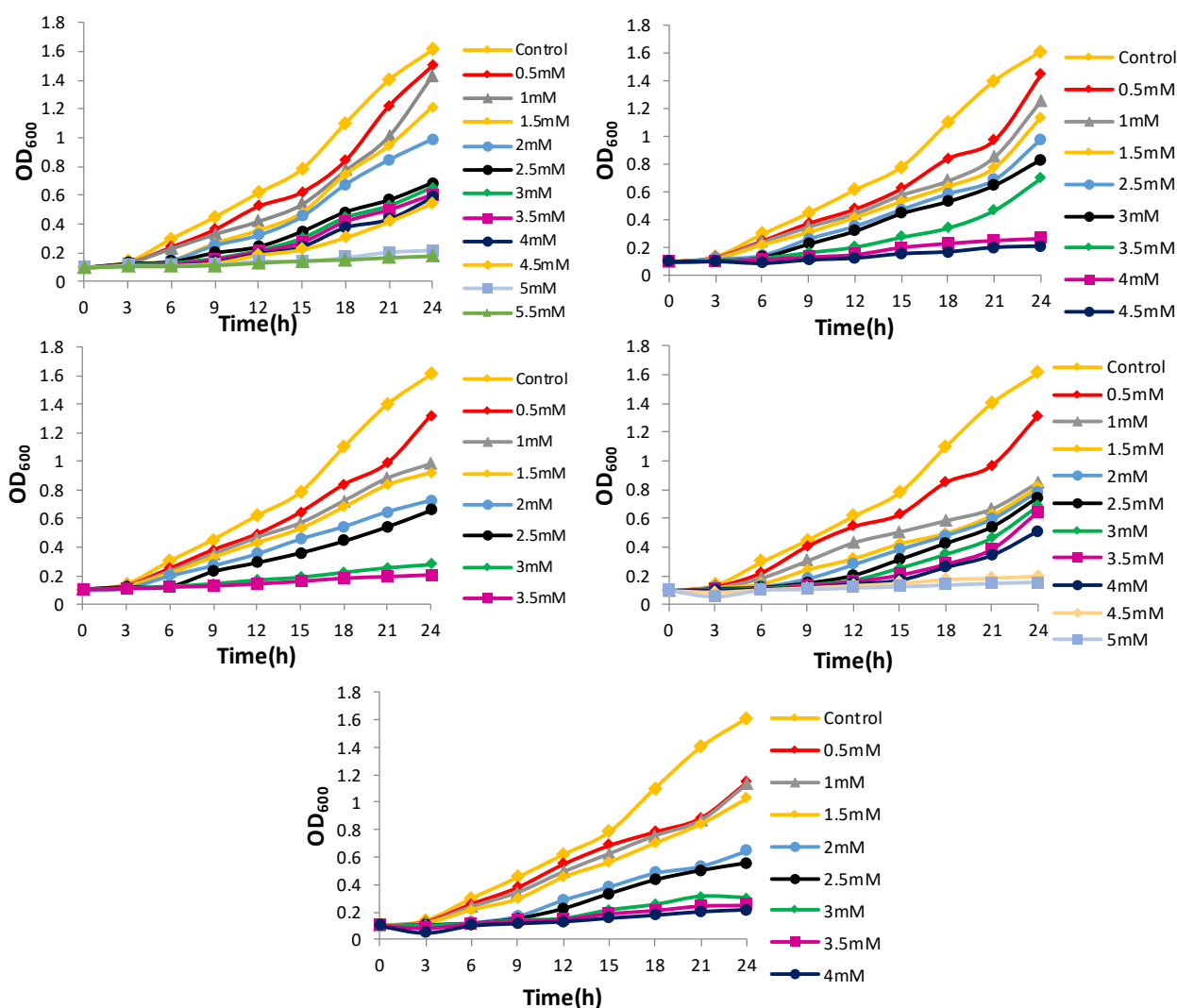
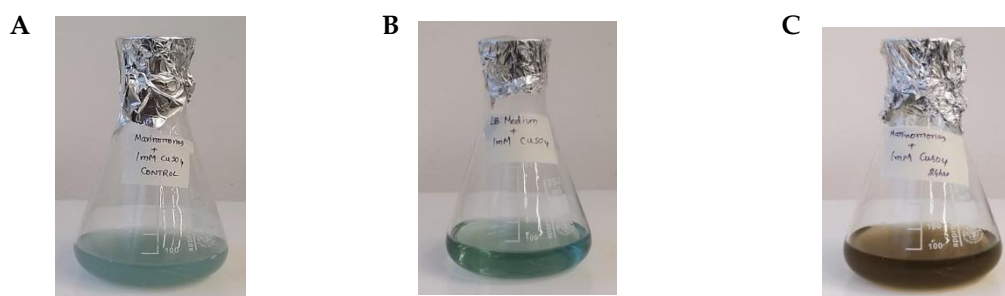
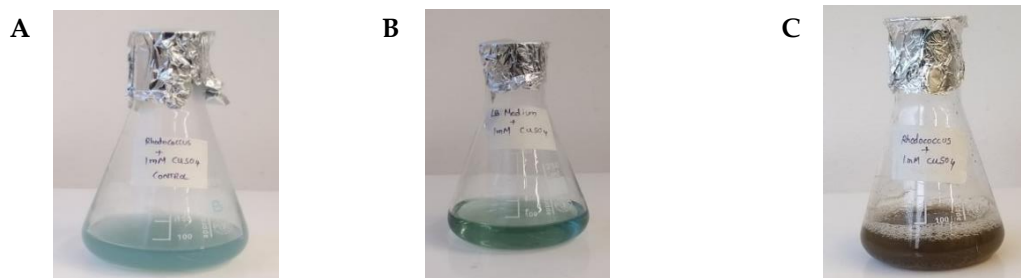


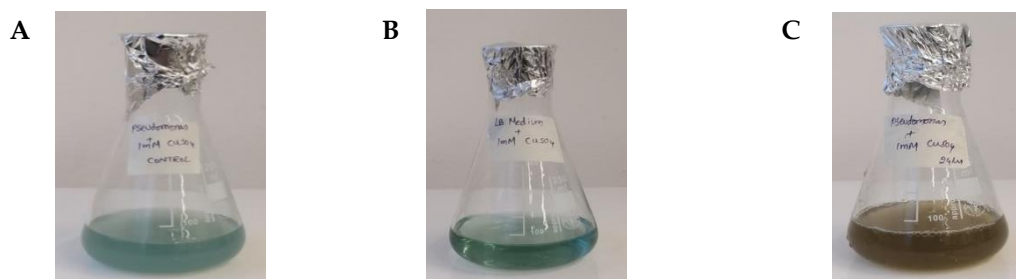
Figure S1. Bacterial growth in the presence of increasing concentration of CuSO₄.



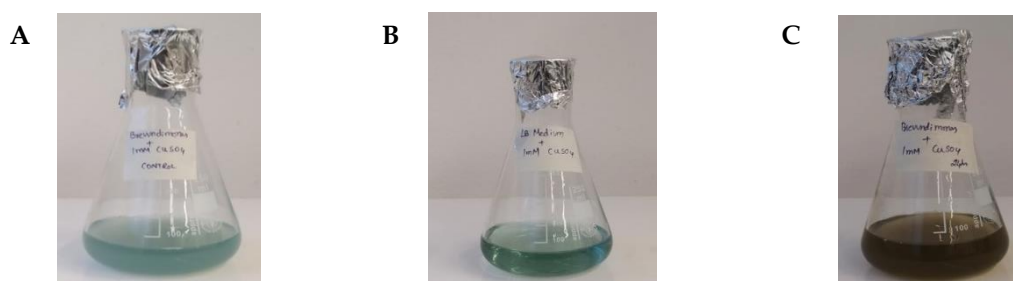
Marinomonas ef1



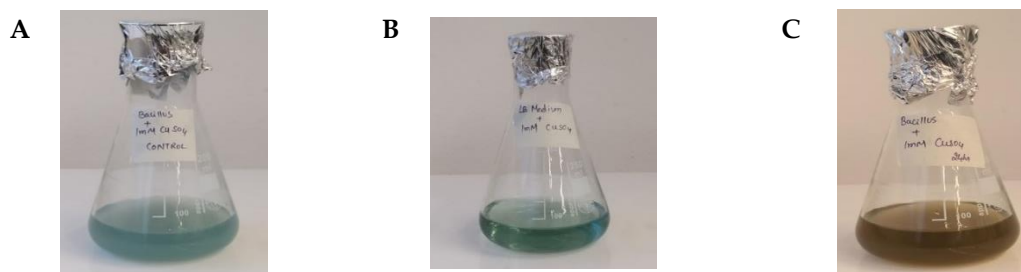
Rhodococcus ef1



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 CuSO₄; **B.** Control with LB medium with 1mM CuSO₄; **C.** Biosynthesized Cu NPs.

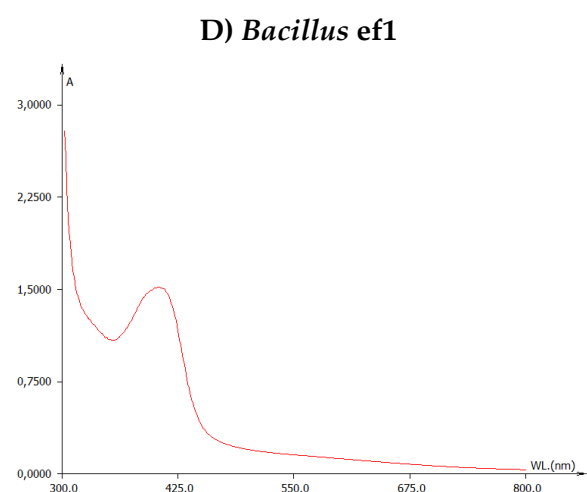
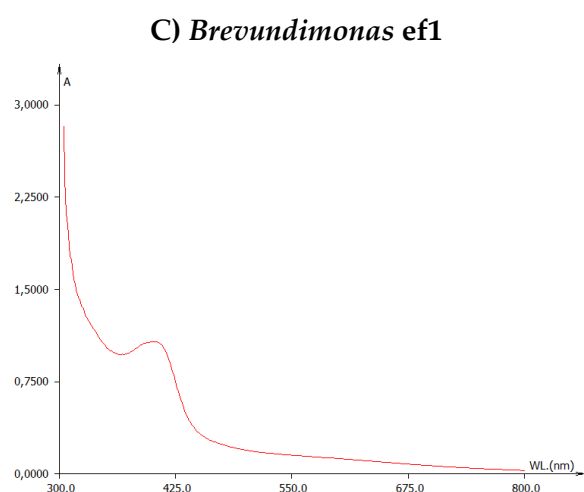
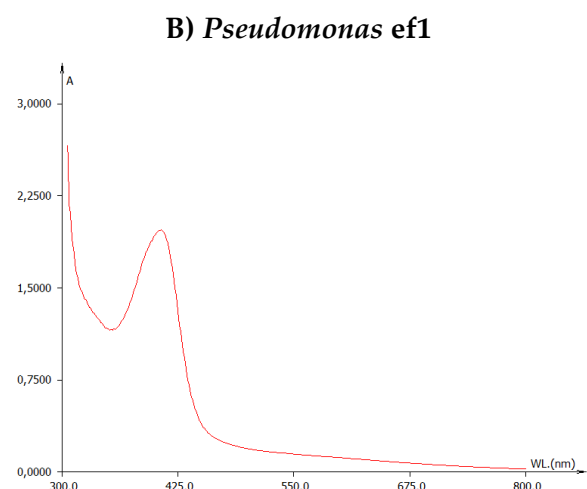
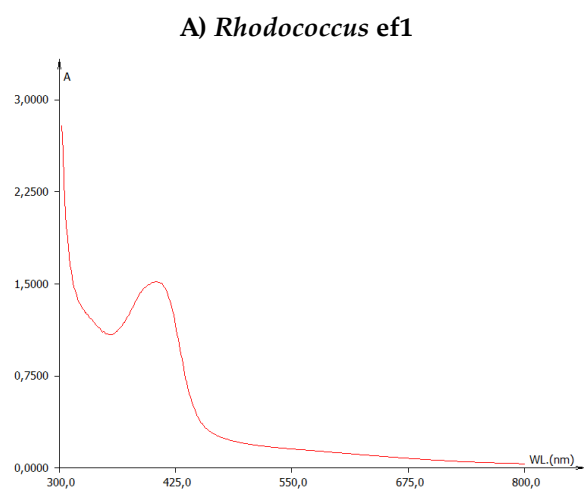


Figure S3. UV-Vis absorbance spectra of bio- CuO NPs synthesized from (A) *Rhodococcus* ef1, (B) *Pseudomonas* ef1, (C) *Brevundimonas* ef1; (D) *Bacillus* ef1; (E) *Marinomonas* ef1.

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 cm^{-1} can be attributed to -OH stretching, the peak at around 3245 cm^{-1} can be attributed to -NH of amide. The band at around 3065 cm^{-1} represents the =C-H stretch of aromatic and unsaturated hydrocarbons; on the other hand the band at around 2960 cm^{-1} is due to the C-H stretching of the aliphatic portion of the molecule around nanoparticles. In the range between 2100 to 1800 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 C=O of amide and also in this region can be possible the observe the bending of the unsaturated C=C bending; in addition, the band at around 1550 cm^{-1} is representative of the N-H bending of the secondary amide. The peak at around 1440–1455 cm^{-1} indicates the bending of the aliphatic -C-H and -CH_3 . The band at around 1390 cm^{-1} can be attributed to the -OH bending. The peak at around 1240 cm^{-1} can be assigned to C-N stretching vibration of amide, and the peak at around 1100 cm^{-1} to the C-O stretching of alcohol. The broad adsorption at around 900–800 cm^{-1} can be due to the rocking vibration out of plane of -NH portion of the amide.

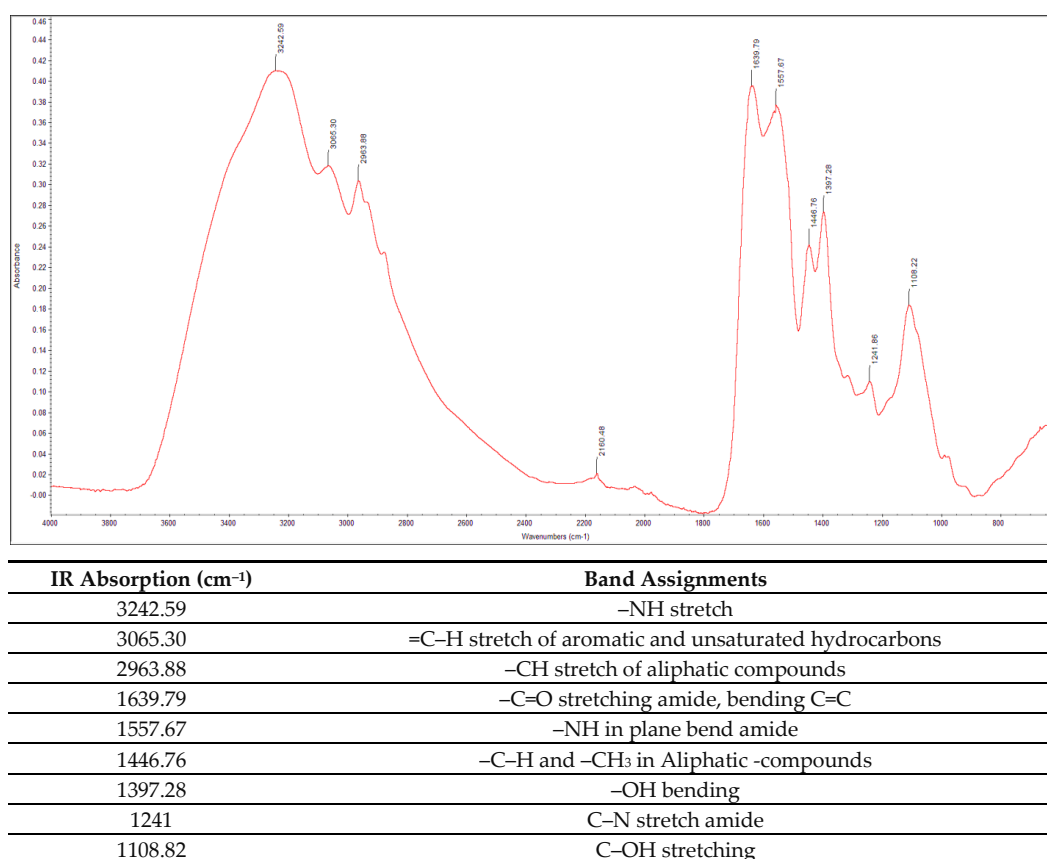
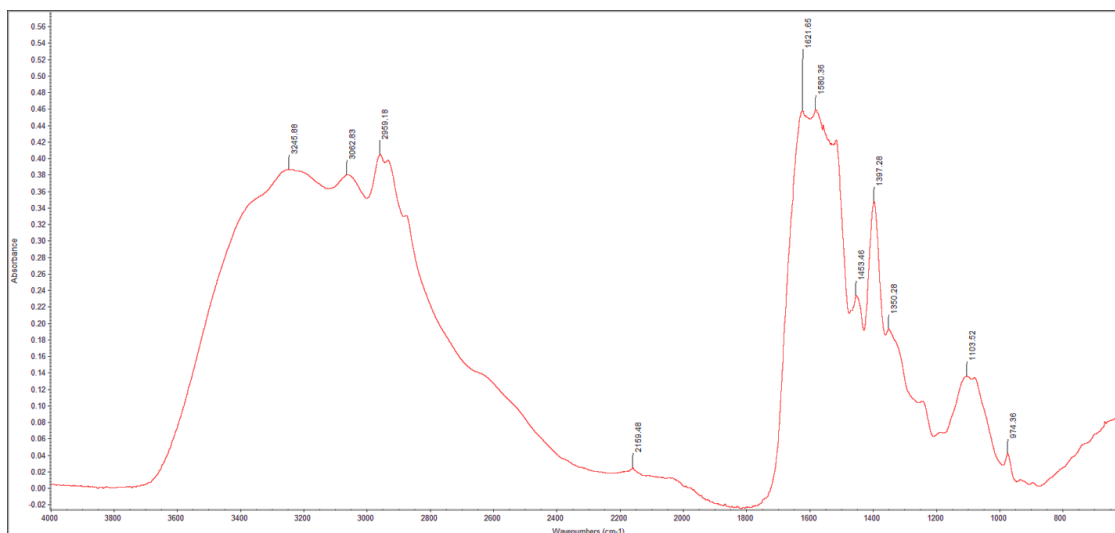
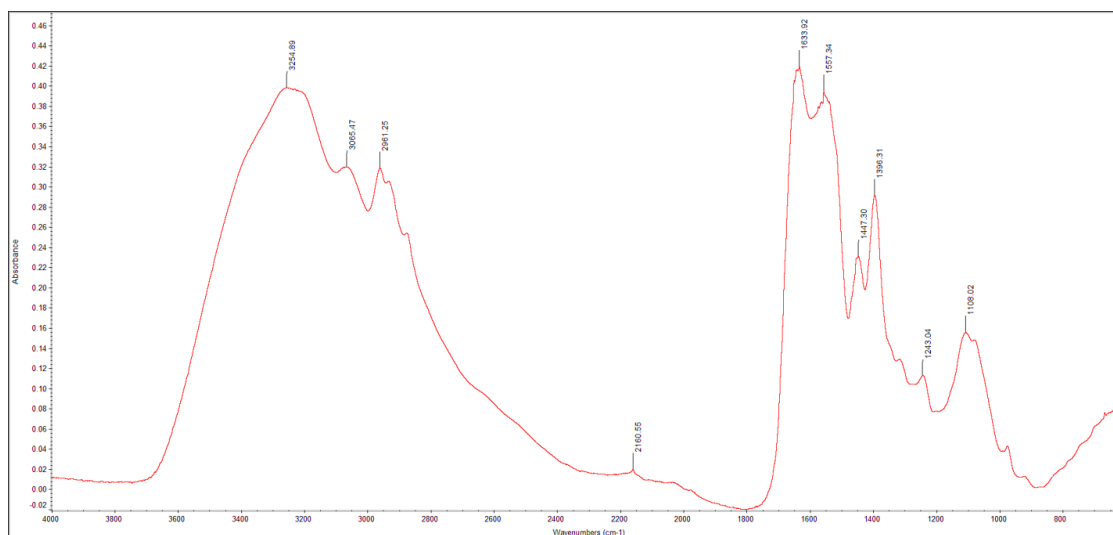


Figure S4. FTIR spectrum of biosynthesized CuO NPs from *Marinomonas ef1* and IR assignments.



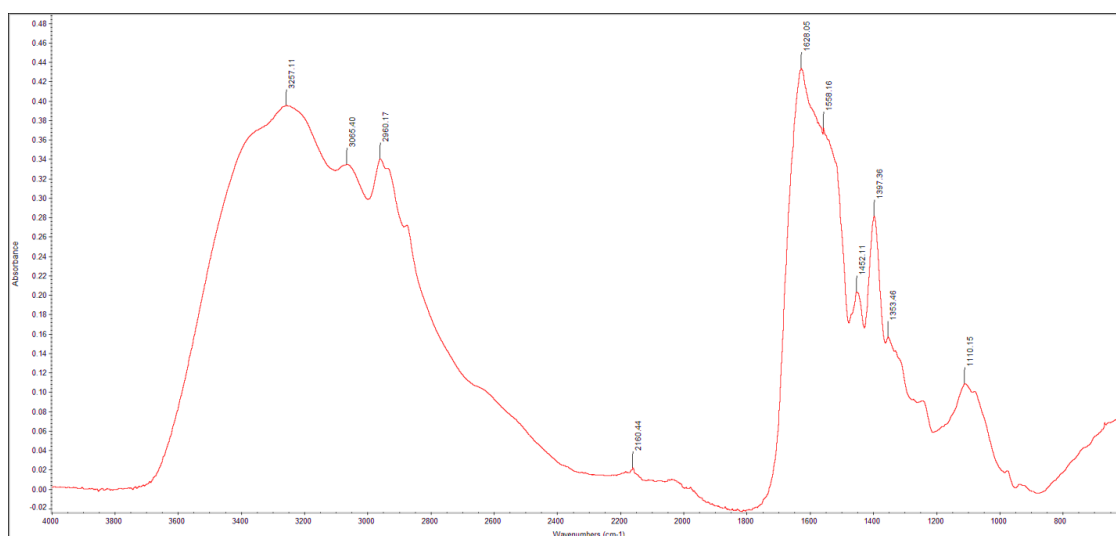
IR Absorption (cm ⁻¹)	Band Assignments
3245.88	-NH stretch
3062.83	=C-H stretch of aromatic and unsaturated hydrocarbons
2959.18	-CH stretch of aliphatic compounds
1621.65	-C=O stretching amide, bending C=C
1580.36	-NH in plane bend amide
1453.46	-C-H and -CH ₃ in Aliphatic -compounds
1397.28	-OH bending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

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



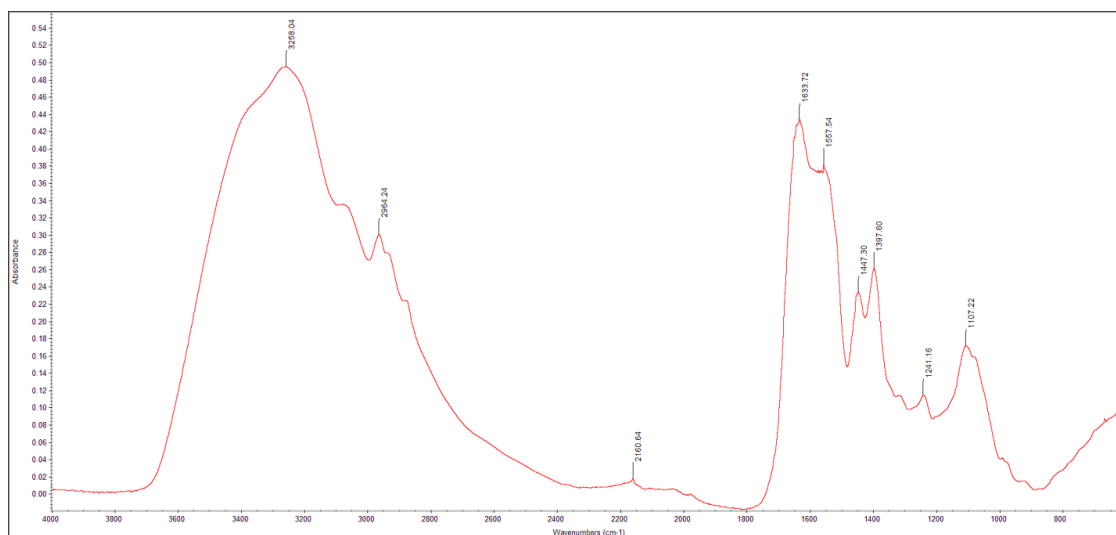
IR Absorption (cm ⁻¹)	Band Assignments
3254.89	-NH stretch
3065.47	=C-H stretch of aromatic and unsaturated hydrocarbons
2961.25	-CH stretch of aliphatic compounds
1633.92	-C=O stretching amide, bending C=C
1557.34	-NH in plane bend amide
1447.30	-C-H and -CH ₃ in Aliphatic -compounds
1396.31	-OH bending
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 ⁻¹)	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 ₃ in Aliphatic -compounds
1397.36	-OH bending
1240.21	C-N stretch amide
1110.15	C-OH stretching

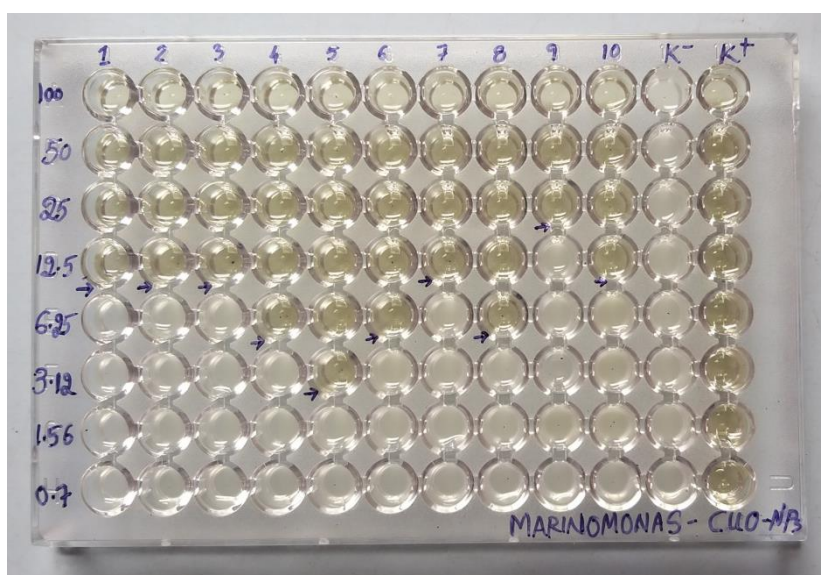
Figure S7. FTIR spectrum of biosynthesized CuONPs from *Brevundimonas* ef1 and IR assignments.



IR Absorption (cm ⁻¹)	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 ₃ in Aliphatic -compounds
1397.60	-OH bending
1241.16	C-OH stretching

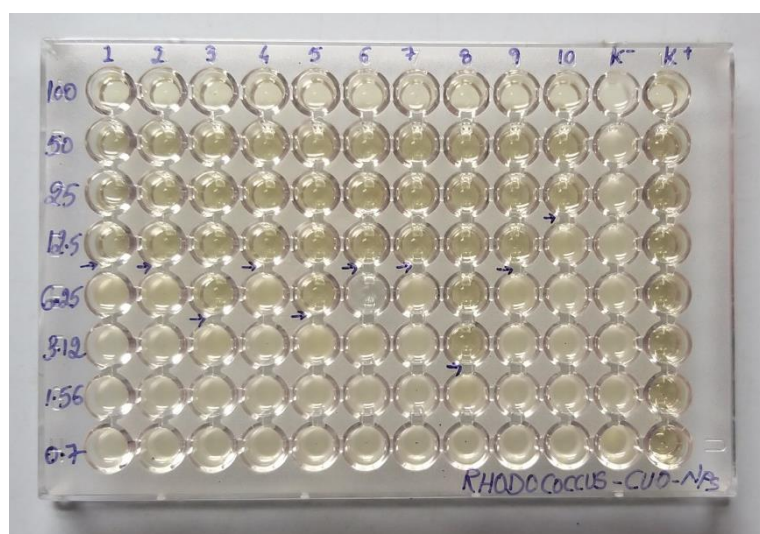
Figure S8. FTIR spectrum of biosynthesized CuO NPs from *Bacillus* ef1 and IR assignments.

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



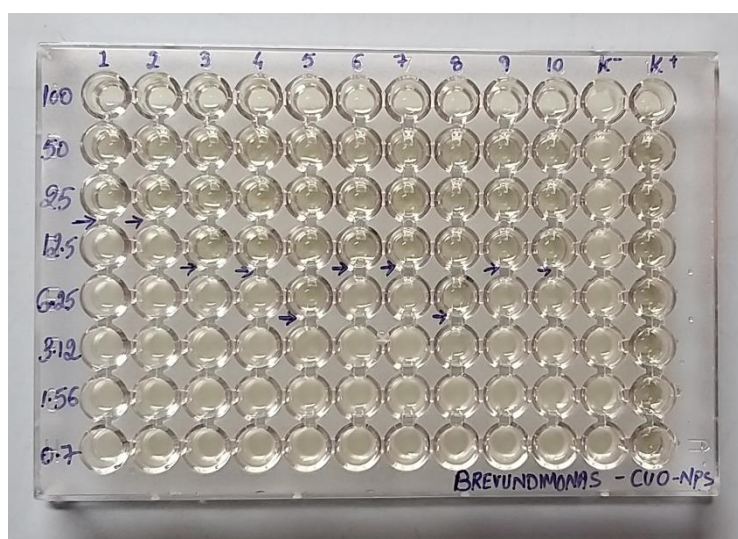
Marinomonas ef1 CuO NPs Broth microdilution method. 1. *Staphylococcus aureus* 2. *Escherichia coli* 3. *Klebsiella pneumoniae* 4. *Pseudomonas* sp. 5. *Proteus mirabilis* 6. *Citrobacter koseri* 7. *Acinetobacter baumannii* 8. *Serratia marcescens* 9. *Candida albicans* 10. *Candida parapsilosis*.

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 $\mu\text{g/mL}$.



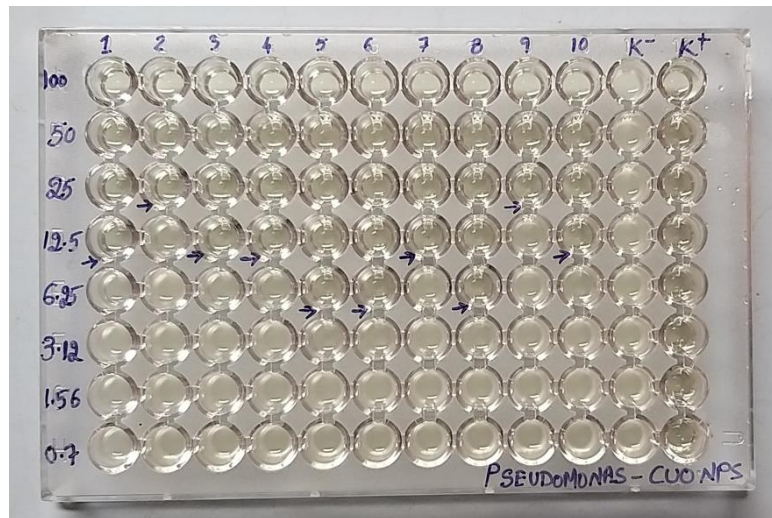
Rhodococcus ef1 CuO NPs Broth microdilution method. 1. *Staphylococcus aureus* 2. *Escherichia coli* 3. *Klebsiella pneumoniae* 4. *Pseudomonas sp.* 5. *Proteus mirabilis* 6. *Citrobacter koseri* 7. *Acinetobacter baumannii* 8. *Serratia marcescens* 9. *Candida albicans* 10. *Candida parapsilosis*.

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.



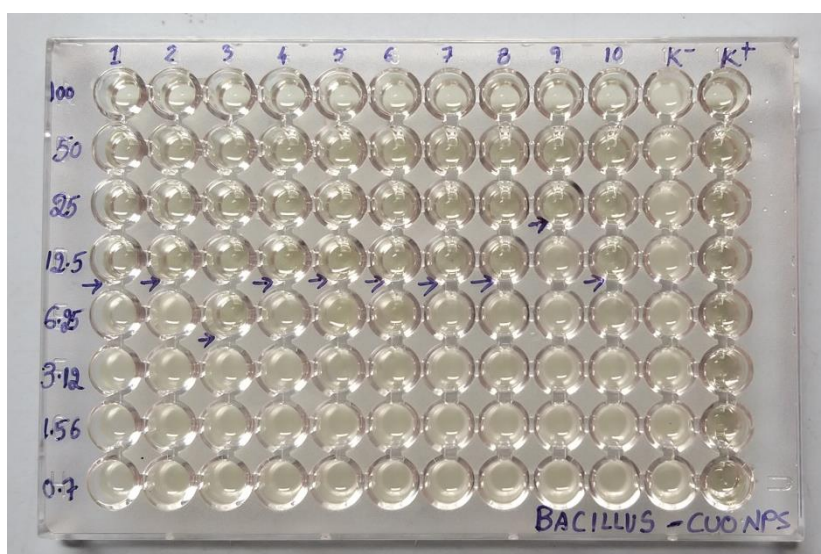
Brevundimonas ef1 CuO NPs Broth microdilution method. 1. *Staphylococcus aureus* 2. *Escherichia coli* 3. *Klebsiella pneumoniae* 4. *Pseudomonas* sp. 5. *Proteus mirabilis* 6. *Citrobacter koseri* 7. *Acinetobacter baumannii* 8. *Serratia marcescens* 9. *Candida albicans* 10. *Candida parapsilosis*.

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 µg/mL), whereas *Escherichia coli*, *Pseudomonas sp.*, *Citrobacter koseri* and *Acinetobacter baumannii* showed a MIC of 12.5 µg/mL. *Escherichia coli* showed a MIC 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 a MIC of 12.5 µg/mL and 6.25 µg/mL respectively. The lowest MBC value among gram-negative bacteria was in *Klebsiella pneumoniae* (6.25 µg/mL). *Escherichia coli*, *Pseudomonas sp.*, *Proteus mirabilis*, *Citrobacter koseri*, *Acinetobacter baumannii* and *Serratia marcescens* showed a MBC of 12.5 µg/mL. Among gram positive bacteria *Staphylococcus aureus* showed a MBC of 12.5 µg/mL. Among Fungi *Candida albicans* and *Candida parapsilosis* showed MBC of 25 µg/mL and 12.5 µg/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*.