

Biosynthesis of silver nanoparticles by the psychrophilic *Rhodoglobus* sp. strain CPHL_1 from Antarctica

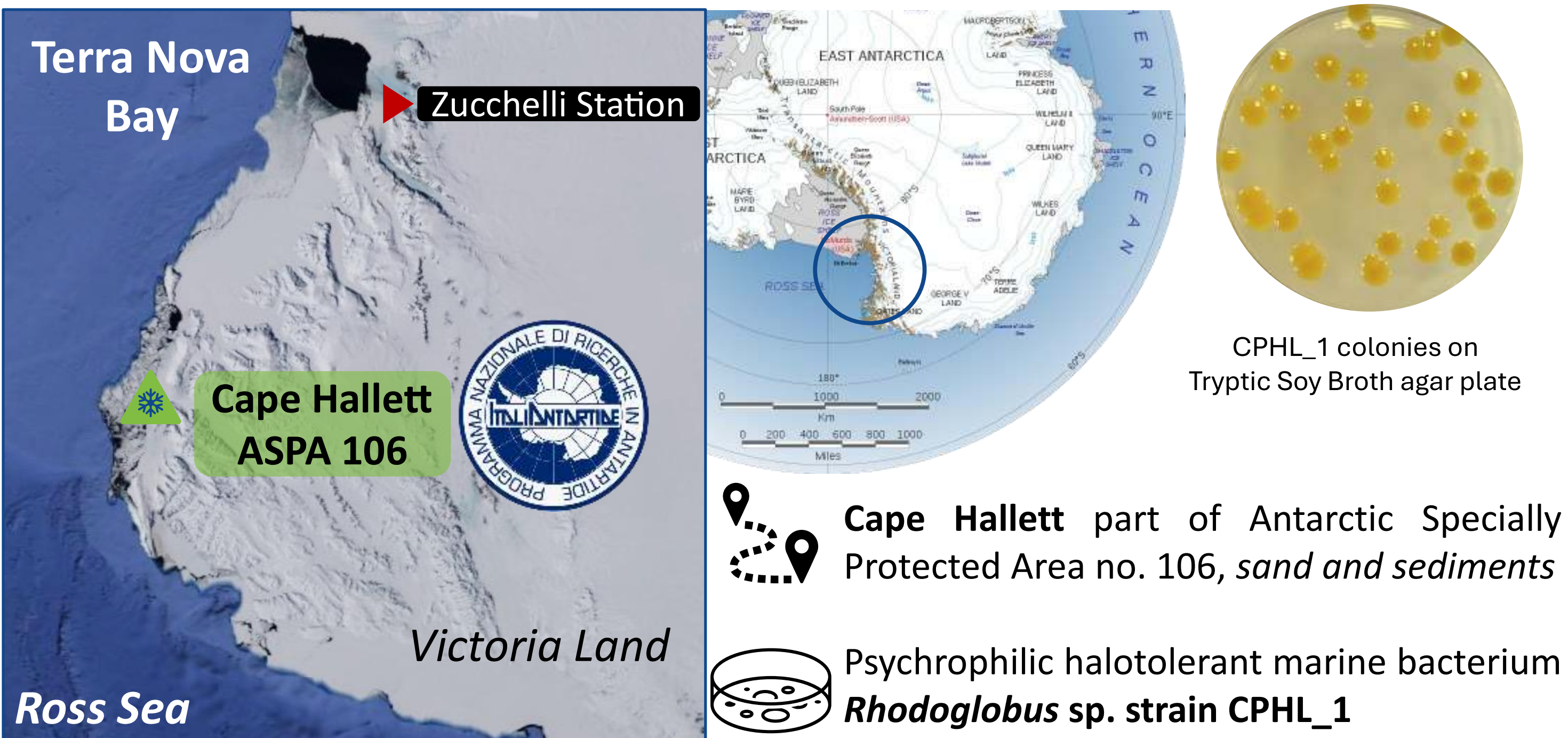
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Rhodoglobus sp. strain CPHL_1 isolated from Antarctica



Antarctic environmental samples collected by ICB extremophiles research group during the XXXIV (2018/19) Italian Antarctic Expedition. Use of oligotrophic media and prolonged incubation to isolate microorganisms from rarely recovered genera.

Strain CPHL_1 is a new *Rhodoglobus* species

Digital DNA-DNA hybridization and Average Nucleotide Identity values

Only other two species with taxonomically valid published names belong to *Rhodoglobus* genus, i.e., *R. aureus* (Reddy *et al.* 2003; An *et al.* 2010) and *R. vestalii* (Sheridan *et al.* 2003).

Genomes compared [ref. CPHL_1]	Accession number	dDDH [confidence interval] (%)	ANIb [aligned nucleotide] (%)	ANIm [aligned nucleotide] (%)
<i>Rhodoglobus aureus</i> JCM 12762	GCA_039527955.1	38.3 [35.9-40.8]	88.9 [70.8]	90.0 [71.3]
<i>Rhodoglobus vestalii</i> DSM 21947	GCA_006788895.1	25.1 [22.8-27.6]	81.7 [60.4]	84.7 [44.0]
<i>Cryobacterium psychrophilum</i> DSM 4854	GCA_004365915.1	23.8 [21.5-26.3]	70.11 [36.9]	86.1 [3.4]
<i>Salinibacterium amurskyense</i> DSM 16400	GCA_002797685.1	23.0 [20.7-25.5]	79.8 [59.7]	84.5 [36.0]
<i>Salinibacterium hongtaonis</i> CGMCC 1.16371	GCA_003065485.1	20.9 [18.7-23.3]	69.9 [31.9]	84.2 [2.6]

Digital DNA-DNA hybridization (dDDH) value was below the 70% species threshold for every species, and the closest being *Rhodoglobus aureus* JCM 12762 (dDDH 38.3%), supporting its proposal as a new species. This classification was corroborated by average nucleotide identity (ANI) analyses, with ANIb and ANIm values in comparison to this strain of 88.9% and 90.0%, respectively, all in agreement with the 95% cutoff for species-level identification

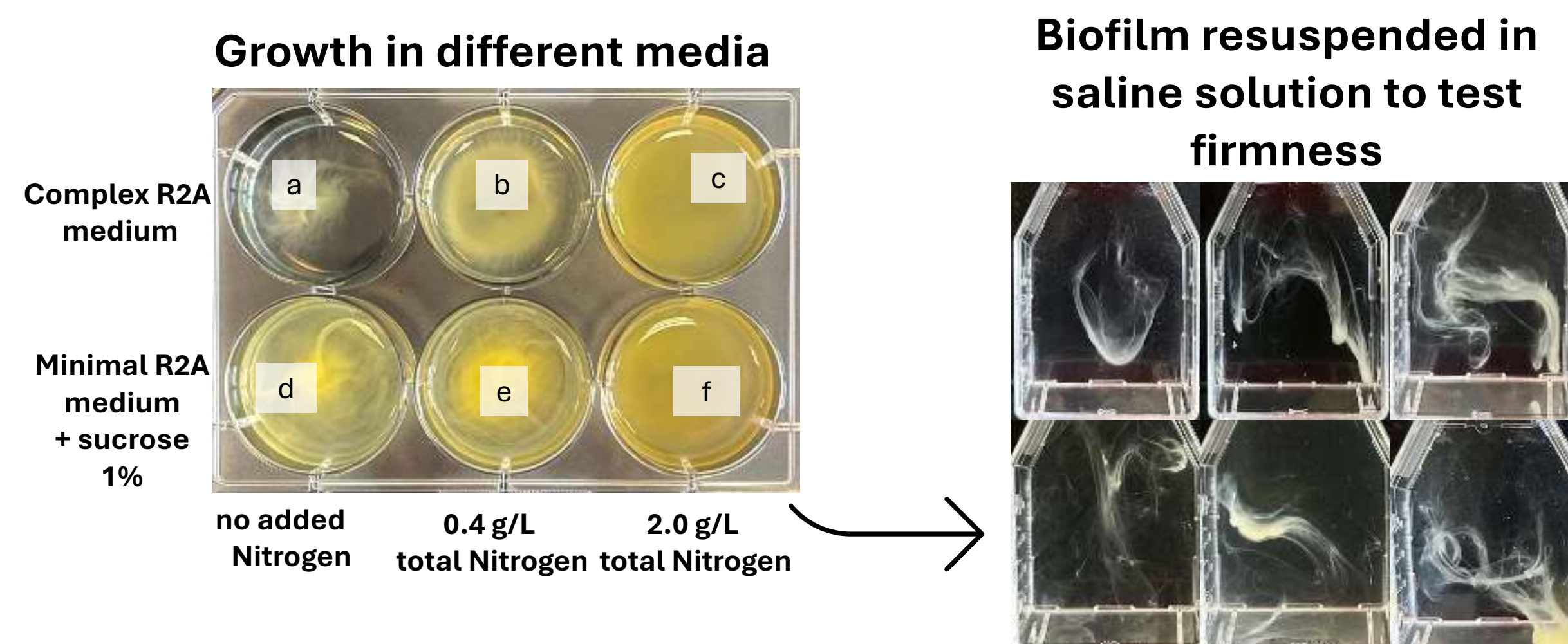
Strain CPHL_1 displayed biofilm-forming abilities

Optimal conditions

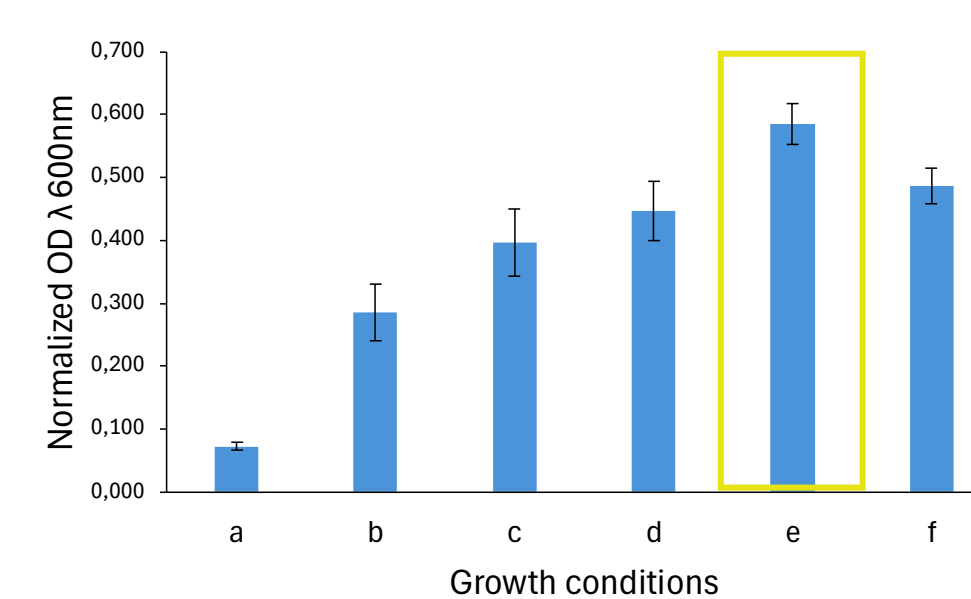
- Temperature: 15 °C
- Salinity: 0.5% NaCl
- pH: 7-8

Some of the biochemical characteristics:

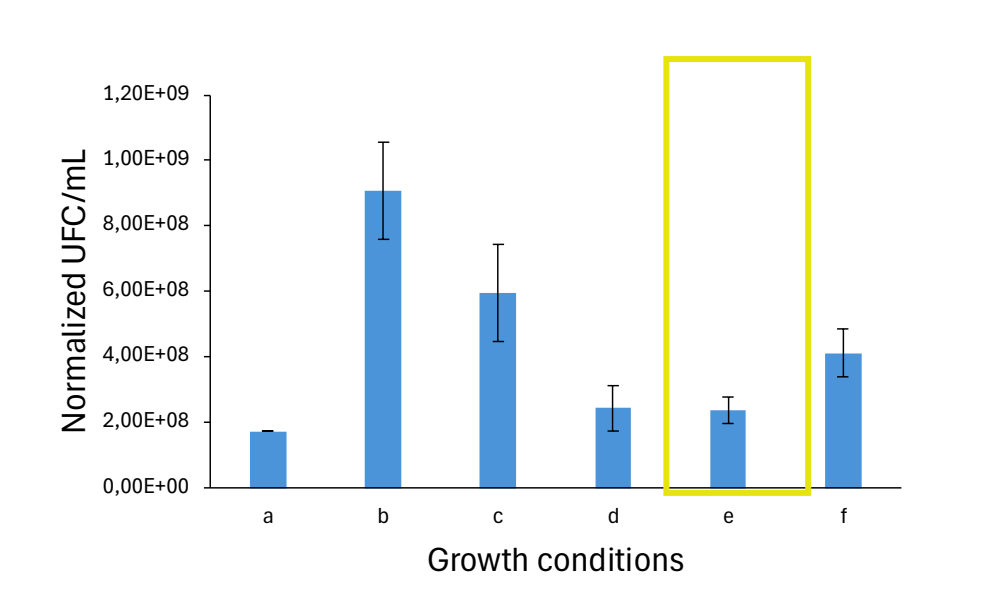
- Catalase positive
- Nitrate reduction positive
- Motility negative
- Sporulation negative
- β-galactosidase positive



Biofilm formation capabilities evaluation



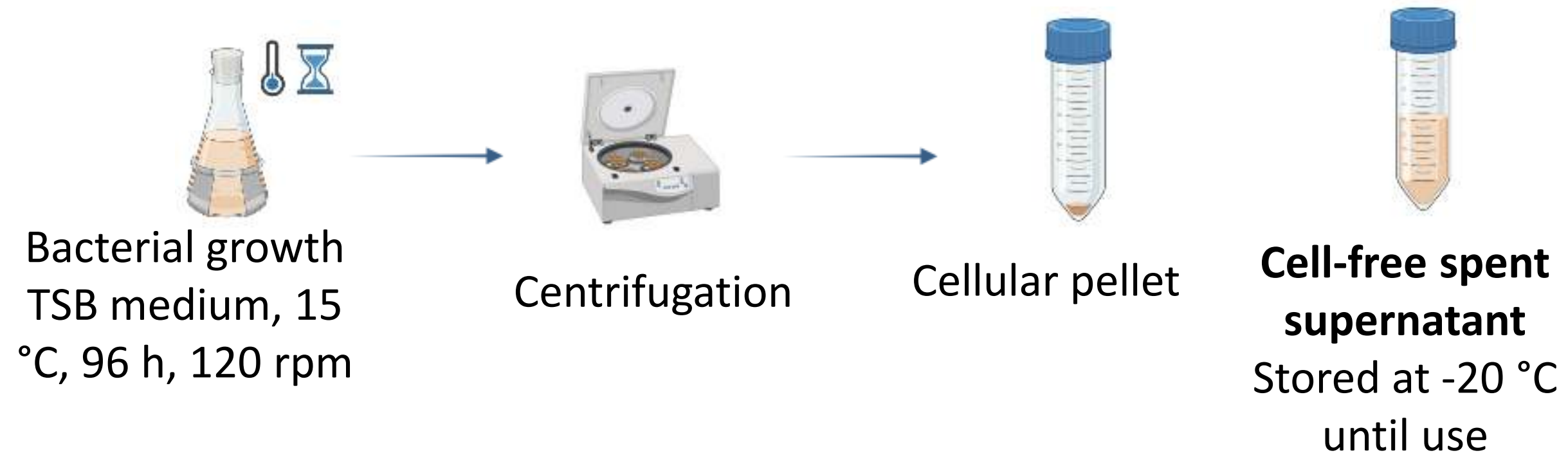
Normalized optical density (λ600 nm) of biofilm resuspended in 10 mL of saline solution.



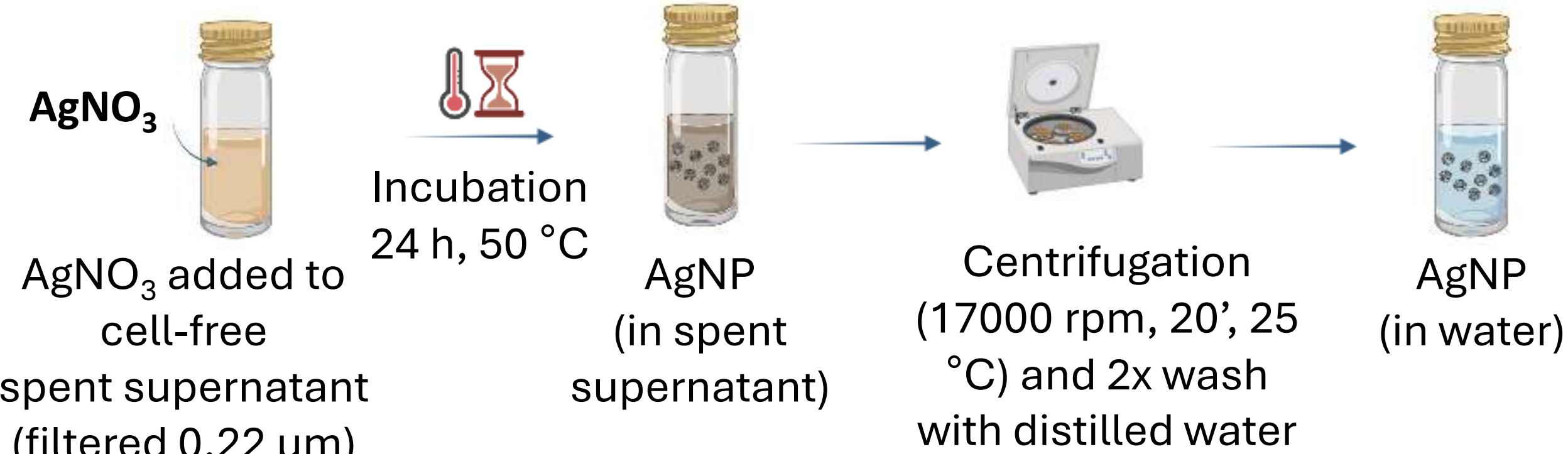
Normalized UFC/mL of biofilm resuspended in 10 mL of saline solution.

Strain CPHL_1 produced silver nanoparticles

Cell-free spent supernatant production



AgNP recovery



Silver nanoparticles application: heavy metal sensing

Preliminary characterization: dynamic light scattering measures

- Size: 62.12 d.nm
- Z-potential: 20.2 mV

Nanoparticles concentration: Beer-Lambert law $c = A/\epsilon d_0$

- A = absorbance at the maximum wavelength (λ436 nm)
- ϵd_0 = molar decadic extinction coefficient

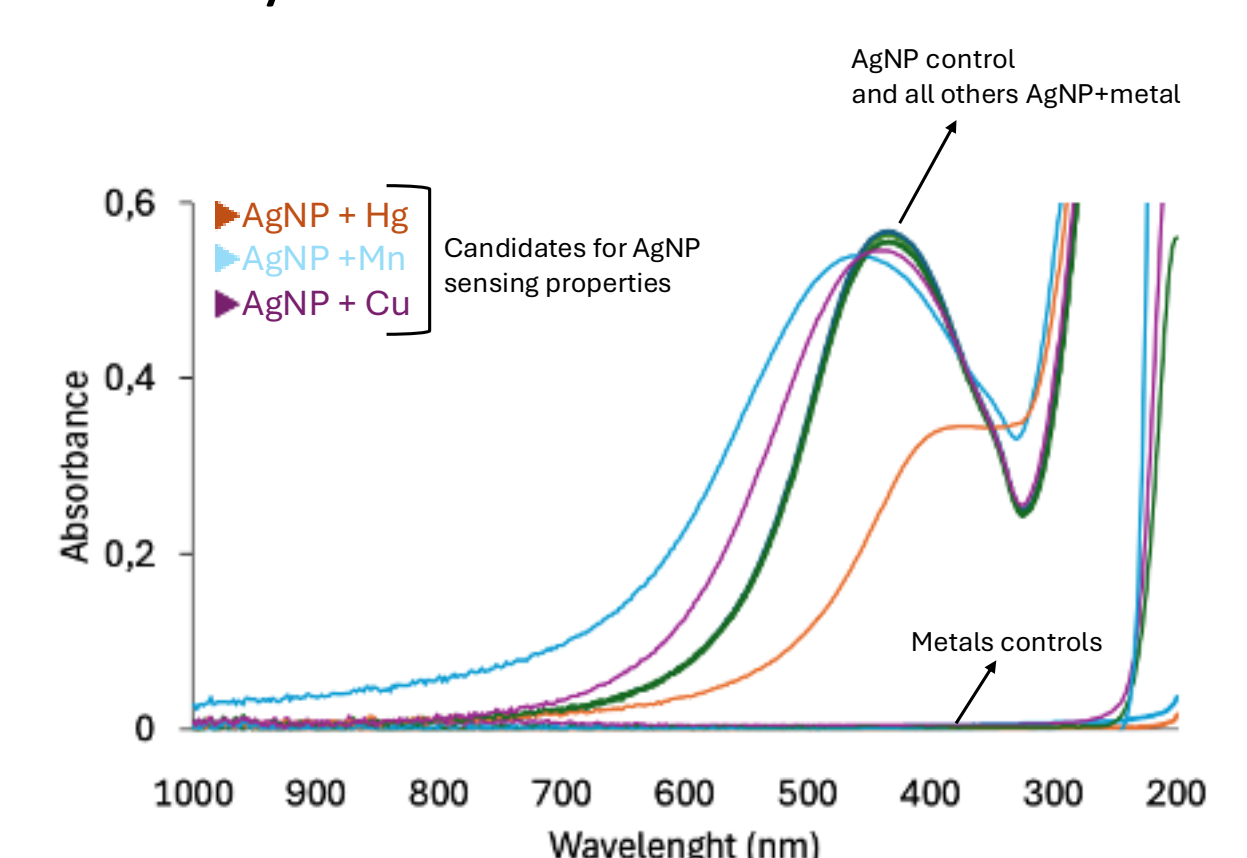
Heavy metals sensing preliminary results

UV-Vis spectra 1000-200 nm of mixtures AgNP plus heavy metals.

Calibration curve for Hg

- Limit of detection: 3.1×10^{-6} M
- Limit of quantification: 9.3×10^{-6} M

LOD = $3.3\sigma/s$
LOQ = $10\sigma/s$
• σ = Residual standard deviation of regression line
• s = slope of calibration curve



References:

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Moretti, M.; Tartaglia, J.; Accotto, G.P.; Beato, M.S.; Bernini, V.; Bevino, A.; Boniotti, M.B.; Budroni, M.; Buzzini, P.; Carrara, S.; et al. Treasures of Italian Microbial Culture Collections: An Overview of Preserved Biological Resources, Offered Services and Know-How, and Management. *Sustainability* 2024, 16, 3777. <https://doi.org/10.3390/su16093777>

GRANTS

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