Beyond the Nanomaterials Approach: Influence of culture conditions on the stability and antimicrobial activity of silver nanoparticles

Supplementary material

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Figure S1. Culture media alter the UV-Vis spectra of the AgNPs.

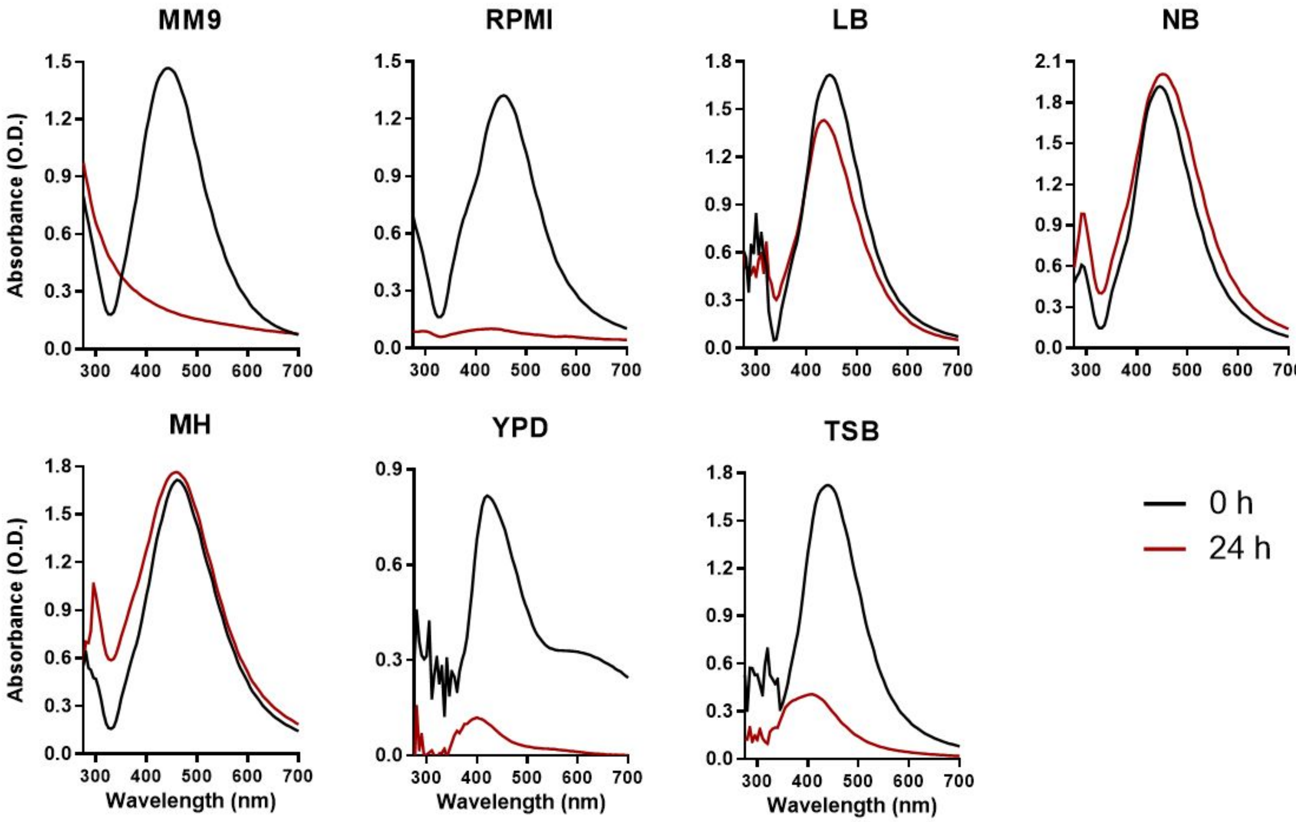

Figure S1. **Culture media alter the UV-Vis spectra of the AgNPs**. UV-Vis spectra profile of AgNPs when exposed to different culture media, at t = 0 h and t = 24 h.

Figure S2. UV-Vis profile from the centrifuged AgNPs supernatant

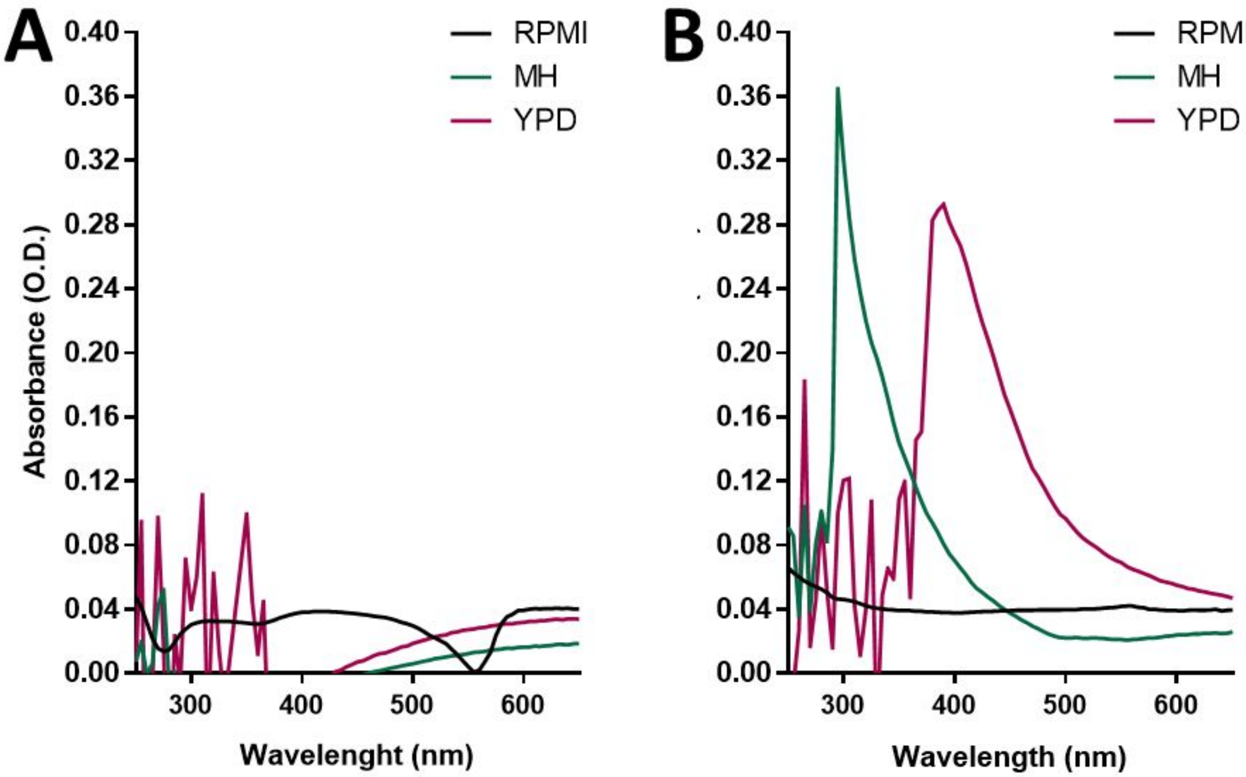

Figure S2. **UV-Vis profile from the centrifuged AgNPs supernatant**. The supernatant of the centrifuged AgNPs in RPMI, MH, and YPD showed different profiles at t = 0 h (A) and t = 24 h (B). In all conditions, the absorbance profile reveals the potential presence of non-AgNPs silver species ʎ<300 nm. Only in YPD t = 24 h, a surface plasmon typical form the AgNPs was observed.

Figure S3. Transmission electron micrographs of AgNPs used in this study.

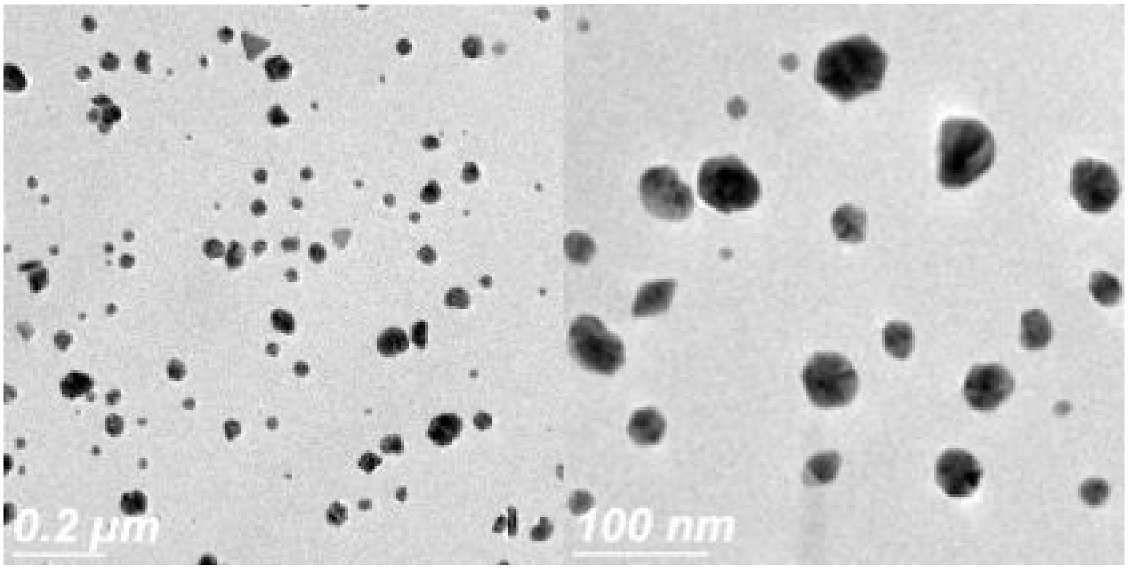

Figure S3. TEM micrographs of silver nanoparticles showed an aspect ratio close to 1, with a metallic core diameter of 35 ±15 nm.

Figure S4. Size distribution of AgNPs in different culture media.

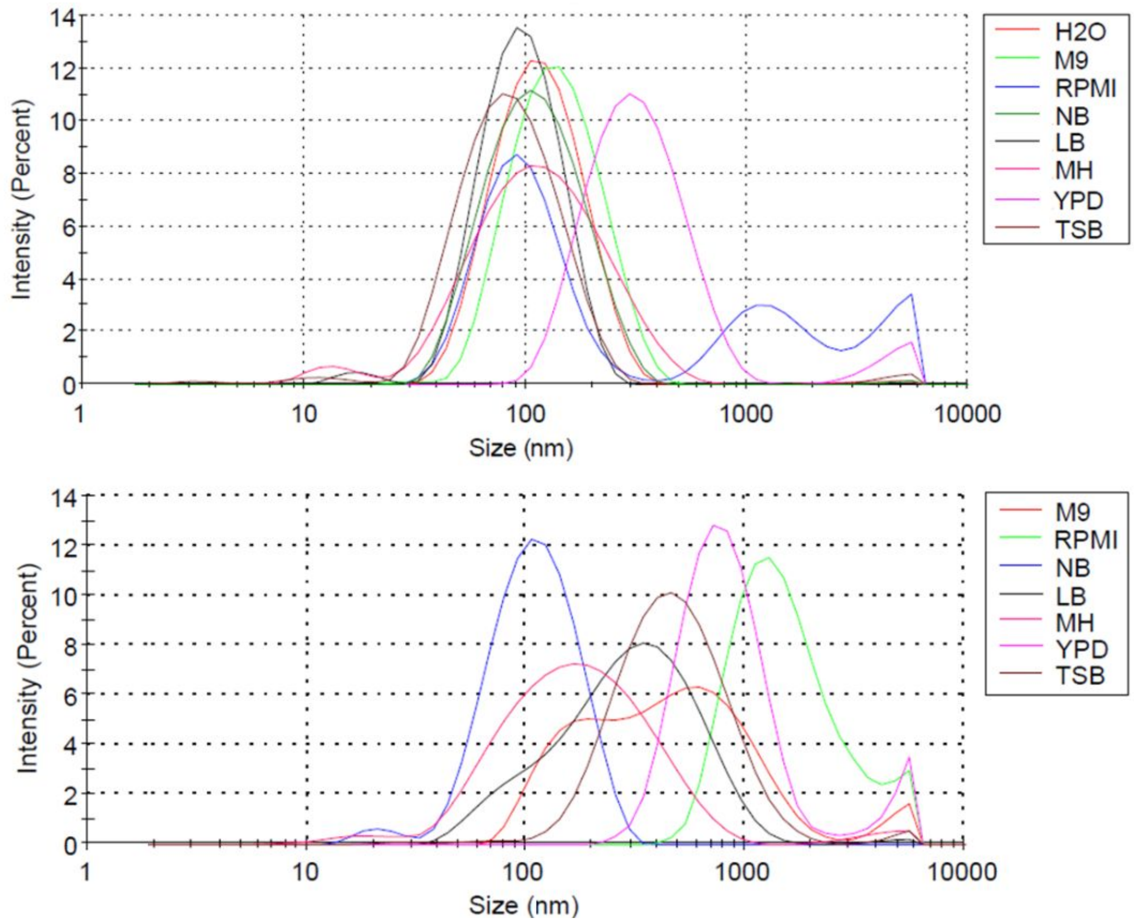

Figure S4. Hydrodynamic Size distribution graphs of AgNPs suspended in different culture media, at t = 0 h (top) and t = 24 h (bottom). The graphs reveal that AgNPs hydrodynamic size changes over time in different culture media.

Table S1. DLS analysis.

Table S1. DLS characterization of AgNPs in different culture media

Culture media	Hydrodynamic size (nm)		Polydispersity Index		ʒ-potential (mV)	
	t= 0 h	t= 24 h	t= 0 h	t= 24 h	t= 0 h	t= 24 h
Milli-Q H_2O	95.3	92.2	0.247	0.296	-14.1	-20.5
M9	124.5	332.4	0.196	0.443	-16.5	-17.9
RPMI	136.2	784.2	0.621	0.327	-16.9	-16
NB	87.8	215.1	0.266	0.222	-14.3	-10.8
LB	77.7	90.1	0.229	0.404	-10	-16.1
MH	89.8	113.6	0.369	0.417	-8.16	-24.4
YPD	299.6	314	0.293	0.384	-16.4	-18.1
TSB	67.4	373.5	0.291	0.315	-14.8	-15.6

Table S2. Culture media broth major components.

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Culture media	Grams per liter of solution (g/L)				pH
	Carbon source	Amino acids/ proteins	Inorganics Salts	Others	
M9	4	0	11.67	Biotin .001 Trace elements Thiamine .001	7.5
RPMI *	2	0.99	9.35	Vitamins 0.04 Glutathione 0.001 Phenol Red 0.0053	7.2
NB	0 #	8	0 #	0 #	6.8
LB	0 #	15	5 #	0 #	7.5
MH	1.5 #	19.5	0 #	0 #	7.4
YPD	20	30	0 #	0 #	6.5
TSB	2.5 #	20	7.5 #	0 #	7.3

#= It can be inherently added by the other components, due to the non-defined composition nature of the culture media's formulations.
* RPMI supplemented with L-Glu+, and NaCOOH-
** Thiamin

Table S3. Silver content estimated by ICP-OES

Table S3. The silver content varies over time in the different culture media

Culture media	Silver content (ppm)	
	t = 0 h	t = 24 h
RPMI	0.34	0.17
MH	1.16	0.89
YPD	1.41	1.70

Table S4. AgNPs MIC on *E. coli*, on different culture media

Table S4. AgNPs MIC on *E. coli*, on different culture media, as determined by our study and the meta-analysis of the literature.

Culture media	MIC ranges (µg ml^{-1})[#]			References
	Our Data	Data from literature: All culture conditions[**]	Data from literature: Similar conditions[***]	
M9	N/A[*]	0.11 – 0.21	0.11 – 0.21	1,2
RPMI 1640	0.5	1.25	1.25	3
LB	1	1 – 40	1 – 40	2,3,13–23
NB	2.5	0.25 - 180	1 – 8	4–12
MH	12.5	0.78 – 31.25	1.6 – 31.25	1,22,24–34
TSB	15	27 – 100	-	22,35
BHI	N/A[*]	0.5 - 2	1.7 – 2	36–38

[#] We provide the MIC values published, still, most reports do not specify if those values are based on the total concentration of AgNPs or only to the silver content.
* The AgNPs MIC values for M9 and BHI were not determined.
** For “All culture conditions” we mean all the MIC values, regardless of initial inoculum size, exposition time, and temperature.
*** For “similar conditions”, we considered those similar to the CLSI A09 protocol with a wider range in the inoculum size: only MIC values, an initial inoculum of 10^5 to 10^6 cells ml^{-1}, 24 h cultures, at 37 °C.

List S1. Culture media broth formulations.

The followed standards recipes for the different culture media are described next. These were used for the preparation of culture media. The quantities of components are for a 1-liter 1X solution and were diluted in 1 distilled water; pH was adjusted using NaOH or HCL, as needed. Culture media was sterilized by autoclaving or filtration, following the seller recommendations.

CULTURE MEDIA REAGENTS	**QUANTITY**
M9 media	
M9 salt solution	11.52 g
• Na_2HPO_4 3.37 mM (7.52 g/L)	
• KH_2PO_4 2.20 mM (3 g/L)	
• NaCl 0.855 mM (0.5 g/L)	
• NH_4Cl 0.935 mM (0.5 g/L)	
Glucose	4 g
1 mM $MgSO_4$	120 mg
0.3 mM $CaCl_2$	33 mg/L
Biotin	1 µg
Thiamin	1 µg
Trace elements	0.1%
pH 7.5	
RPMI 1640 (R0883)	
Inorganic Salts	9.75 g
• Calcium Nitrate • $4H_2O$ (0.1 g)	
• Magnesium Sulfate (anhydrous) (0.05 g)	
• Potassium Chloride (0.4 g)	
• Sodium Bicarbonate (2 g)	
• Sodium Chloride (6 g)	
• Sodium Phosphate Dibasic (anhydrous) (0.8 g)	
Amino Acids	0.69403 g
• L-Alanyl-L-Glutamine (0)	
• L-Arginine (0.2)	
• L-Asparagine (anhydrous) (0.05)	
• L-Aspartic Acid (0.02)	
• L-Cystine •2HCl (0.0652)	
• L-Glutamic Acid (0.02)	
• L-Glutamine (—)	
• Glycine (0.01)	
• L-Histidine (0.015)	
• Hydroxy-L-Proline (0.02)	
• L-Isoleucine (0.05)	
• L-Leucine (0.05)	

- L-Lysine •HCl (0.04)
- L-Methionine (0.015)
- L-Phenylalanine (0.015)
- L-Proline (0.02)
- L-Serine (0.03)
- L-Threonine (0.02)
- L-Tryptophan (0.005)
- L-Tyrosine •2Na •2H_2O (0.02883)
- L-Valine (0.02)

Vitamins 0.042655

- D-Biotin 0.0002
- Choline Chloride 0.003
- Folic Acid 0.001
- myo-Inositol 0.035
- Niacinamide 0.001
- p-Aminobenzoic Acid 0.001
- D-Pantothenic Acid (hemicalcium) 0.00025
- Pyridoxine • HCl 0.001
- Riboflavin 0.0002
- Thiamine • HCl 0.001
- Vitamin B12 0.000005

Other

- D-Glucose 2
- Glutathione (reduced) 0.001
- Phenol Red •Na 0.0053
- L-Glutamine 0.3
- Sodium Bicarbonate 0

pH 7.2 ±0.2

Nutrient Broth (NB)

Beef Extract	3 g
Peptone	5 g

pH 6.8 ±0.2

Luria Bertani broth (LB)

Peptone	10 g
Yeast Extract	5 g
Sodium Chloride	5 g

pH 7.5 ±0.2

MH

Acid Digest of Casein	17.5 g
Soluble Starch	1.5 g
Beef Extract	2 g

pH 7.4 ±0.2

YPD

Yeast extract	10 g
Peptone	20 g
Dextrose (glucose)	20 g
pH 6.5 ±0.2	

TSB

Pancreatic Digest of Casein	17 g
Sodium Chloride	5 g
Papaic Digest of Soybean Meal	3 g
Dextrose	2.5 g
Dipotassium Phosphate	2.5 g
pH 7.3 ±0.2	

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