Supporting Information

Hierarchical Passage of Gold Nanoclusters in Living Bacteria

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This supporting information includes:

Experimental Set up

Culture of *Lactobacillus rhamnosus*

The *Lactobacillus rhamnosus* were grown in Lactobacillus MRS broth medium, at 1% inoculum incubated for 48 h at 37 °C at 180 rpm.

Growth curve of *Lactobacillus rhamnosus*

The doubling time of the *Lactobacillus rhamnosus* was determined by measuring the optical density of the bacteria after an interval of 3 h for a duration of 48 h. The analysis was carried out by taking the logarithmic values of the optical density against the different time points. The doubling time was found to be 1.1 h.

Synthesis of gold nanoclusters

The gold nanocluster synthesis was carried out by taking 10^9 number of *Lactobacillus rhamnosus* from cultures grown for 48 h at 37 °C in MRS growth medium. The so-obtained bacteria were washed thrice using double-autoclaved deionised water by repeated centrifugation (10 K rpm for 5 min) and re-dispersion to remove the medium completely. To the re-dispersed culture, 2 mM of $HAuCl_4$ was added followed by addition of 0.11 M mercaptoropionic acid as a reducing agent. This reaction mixture was incubated at 37 °C for 3 min followed by centrifugation at 10 K rpm for 5 min to remove the unreacted products. The pellet was re-dispersed in double-autoclaved deionised water for further characterization studies.

Sample preparation for characterization studies

The control bacteria, gold nanocluster bearing bacteria and the progenies were centrifuged at 10K rpm for 5 min and then were re-dispersed in double autoclaved deionised water. The process was performed thrice. The obtained samples were diluted and further used for the characterization studies.

Determination of Colony Forming Unit/mL before and after synthesis

The quantification of bacteria before and after synthesis was carried out by taking *Lactobacillus rhamnosus* from late log phase followed by serially diluting the culture and spreading on MRS agar plates. These agar plates were incubated for 48 h at 37 °C and then the colonies of bacteria were counted. The cfu/mL of *Lactobacillus rhamnosus* was found to be 10^9 cfu/mL. The similar procedure was followed to calculate the cfu/mL after synthesis; it was found to be 10^8 cfu/mL.

Spectroscopic analysis

The UV-visible spectroscopy analysis was carried out in Jasco V-630 spectrophotometer. The fluorescence spectroscopy analysis was done using Fluoromax-4 (Horiba Jobin Yvon). The excitation wavelength used was 320 nm for all the samples.

Field Emission Transmission Electron Microscopy, Energy Dispersive X-ray analysis and Elemental mapping analysis

The diluted samples were drop cast on carbon coated copper mesh and were allowed to dry. The analysis as carried out in Jeol 2100F FETEM. The EDX and elemental mapping analyses were also performed to confirm the presence of Au.

Field Emission Scanning Electron Microscopy

The samples were drop cast on aluminium foil for the analysis and were dried. The analysis was performed in Jeol JSM-7610.

Atomic Force Microscopy

The samples were diluted using double autoclaved deionised water and were drop cast on clean glass slide for drying. The analysis was carried out in Oxford Cypher S atomic force microscope.

Confocal Laser Scanning Microscopy

The prepared samples were imaged by CLSM (Zeiss, LSM 880) using laser at 405 nm.

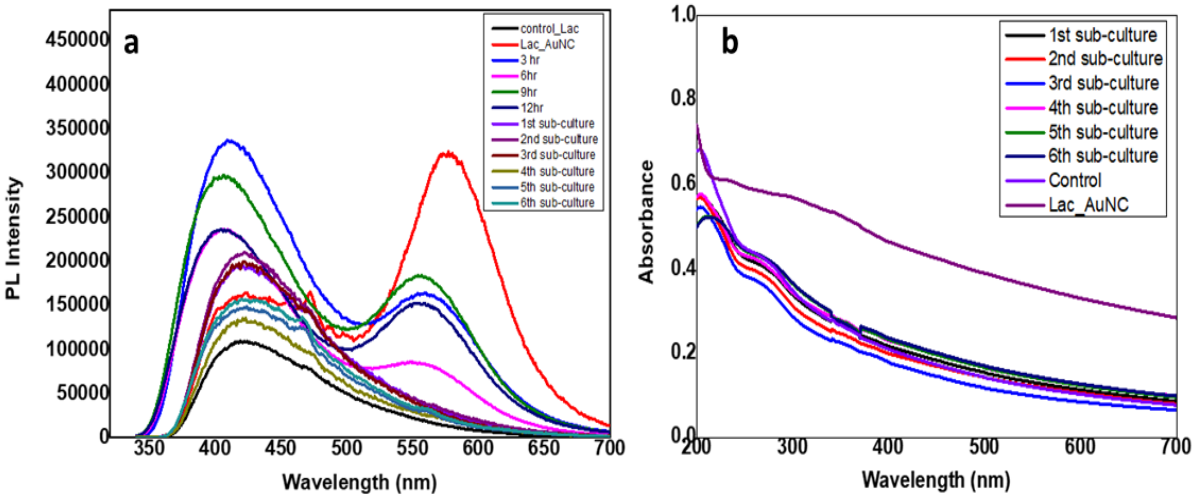


Figure S1. Spectroscopic analysis (a) Photoluminescence spectra, (b) UV absorbance spectra of control, AuNC embedded bacteria (Lac_AuNC) and their progenies collected after 3 h, 6 h, 9 h, 12 h and 1st to 6th subcultures.

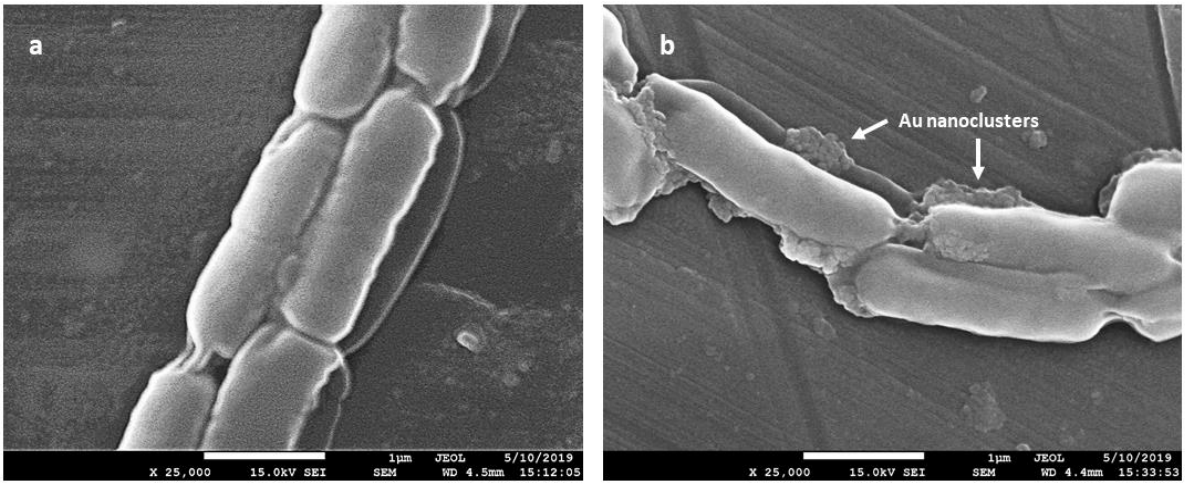


Figure S2. Field emission scanning electron microscopy images of (a) control *Lactobacillus rhamnosus* (Lac_AuNC) bacteria and (b) gold nanocluster bearing *Lactobacillus rhamnosus* (Lac_AuNC).

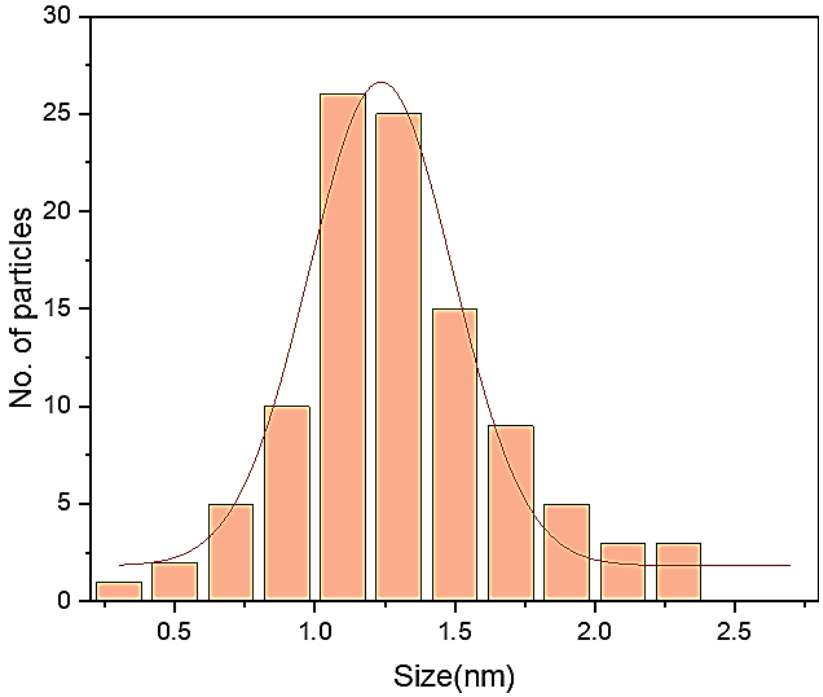


Figure S3. Size distribution. Average size of gold nanoclusters formed on *Lactobacillus rhamnosus* (Lac_AuNC; 1.31±0.36 nm). The size distribution calculation was done using several TEM images and involving about 100 particles.

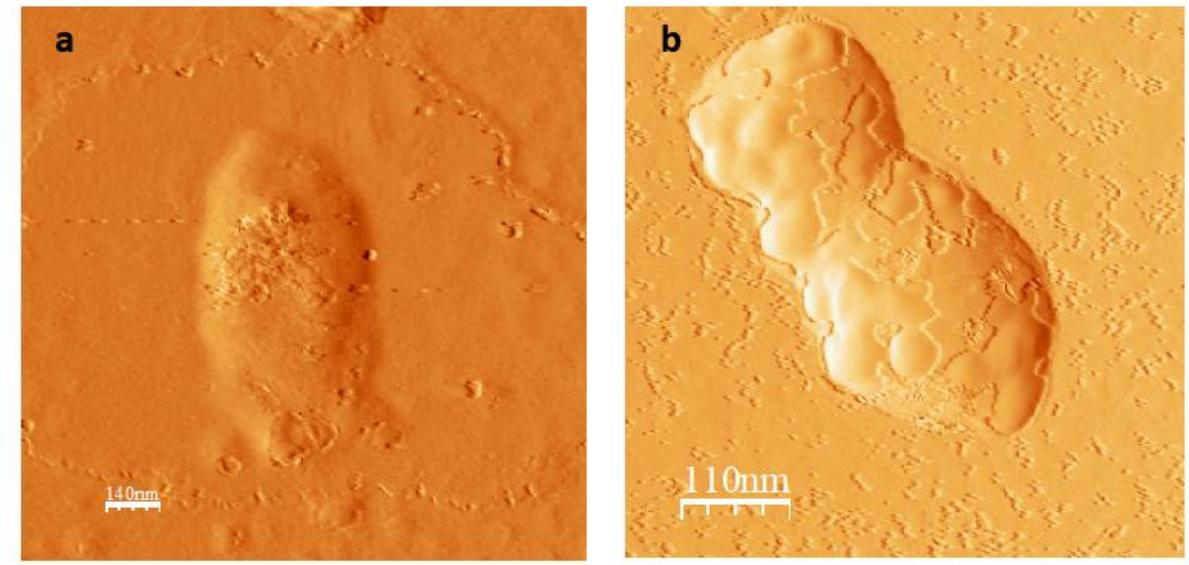


Figure S4. Atomic force microscopy images of (a) control *Lactobacillus rhamnosus* (Lac_AuNC) bacteria and (b) gold nanocluster bearing *Lactobacillus rhamnosus* (Lac_AuNC).

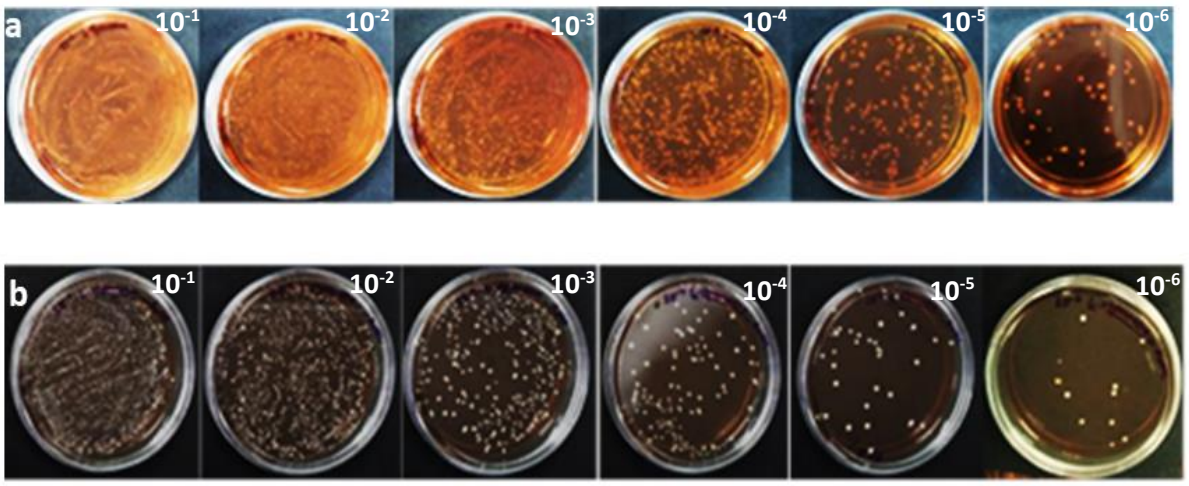


Figure S5. Colony forming unit/mL (a) MRS agar plates containing bacterial colonies obtained after plating serial dilutions (as described in the legends) of control *Lactobacillus rhamnosus* (10^9 CFU/mL) and (b) colonies obtained after plating serial dilutions of gold nanocluster bearing *Lactobacillus rhamnosus* (Lac_AuNC; 10^8 CFU/mL).

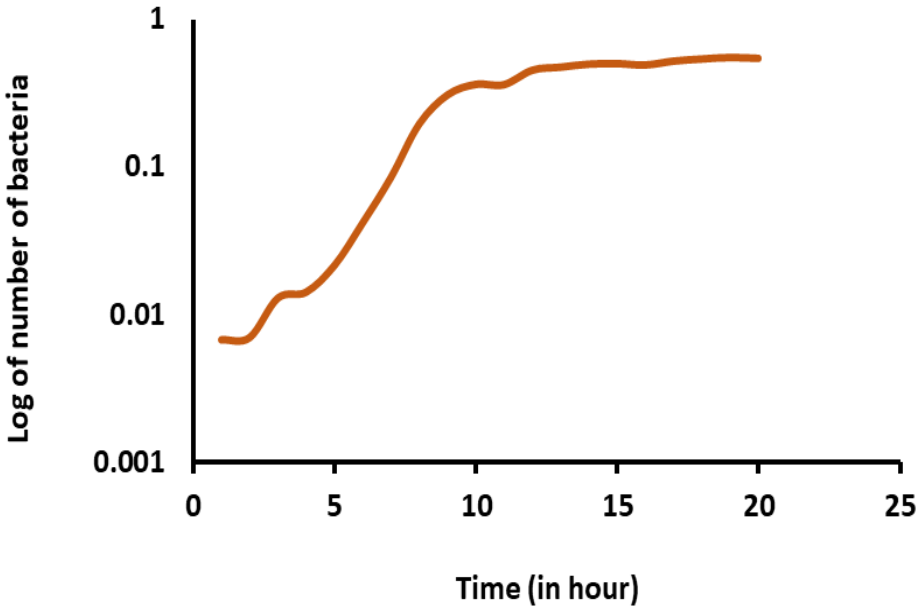


Figure S6. Growth curve. Growth pattern of *Lactobacillus rhamnosus* grown at 37 °C for 48 h of incubation.

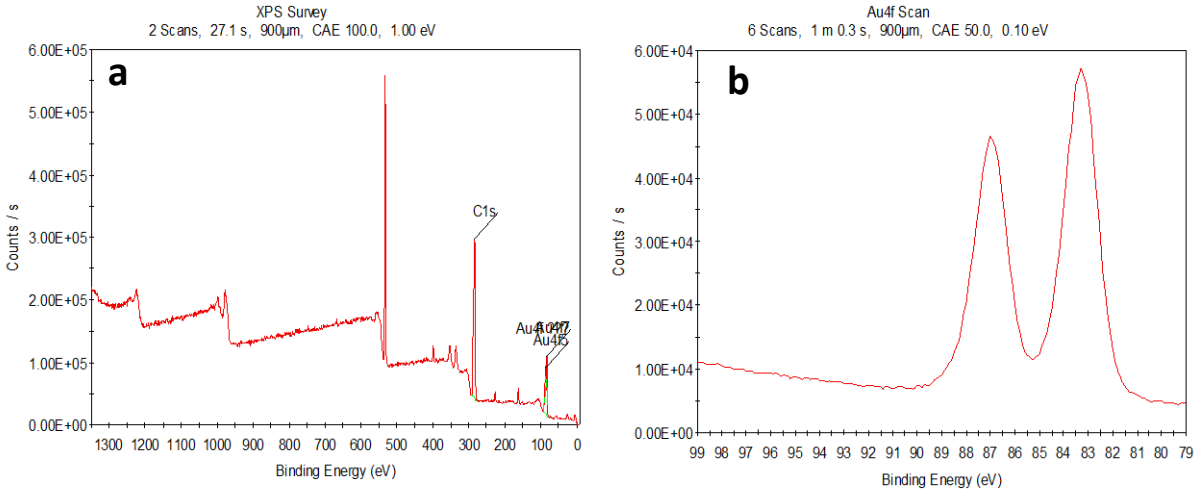


Figure S7. X-ray photoelectron spectroscopy analysis. (a) XPS survey spectrum and (b) Au4f7/2 and Au4f5/2 XPS spectra of gold nanocluster bearing *Lactobacillus rhamnosus*(Lac_AuNC)**.**

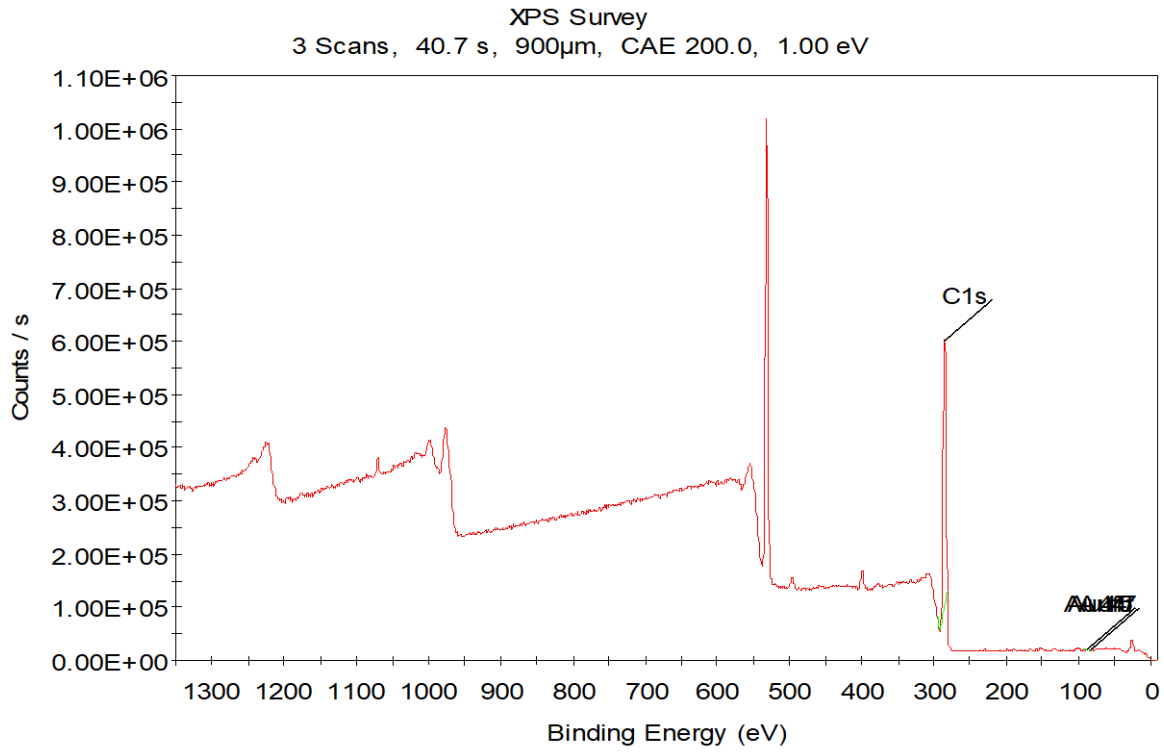


Figure S8. X-ray photoelectron spectroscopy analysis. XPS survey spectrum of progeny bacteria grown after 1st subculture of Lac_AuNC.

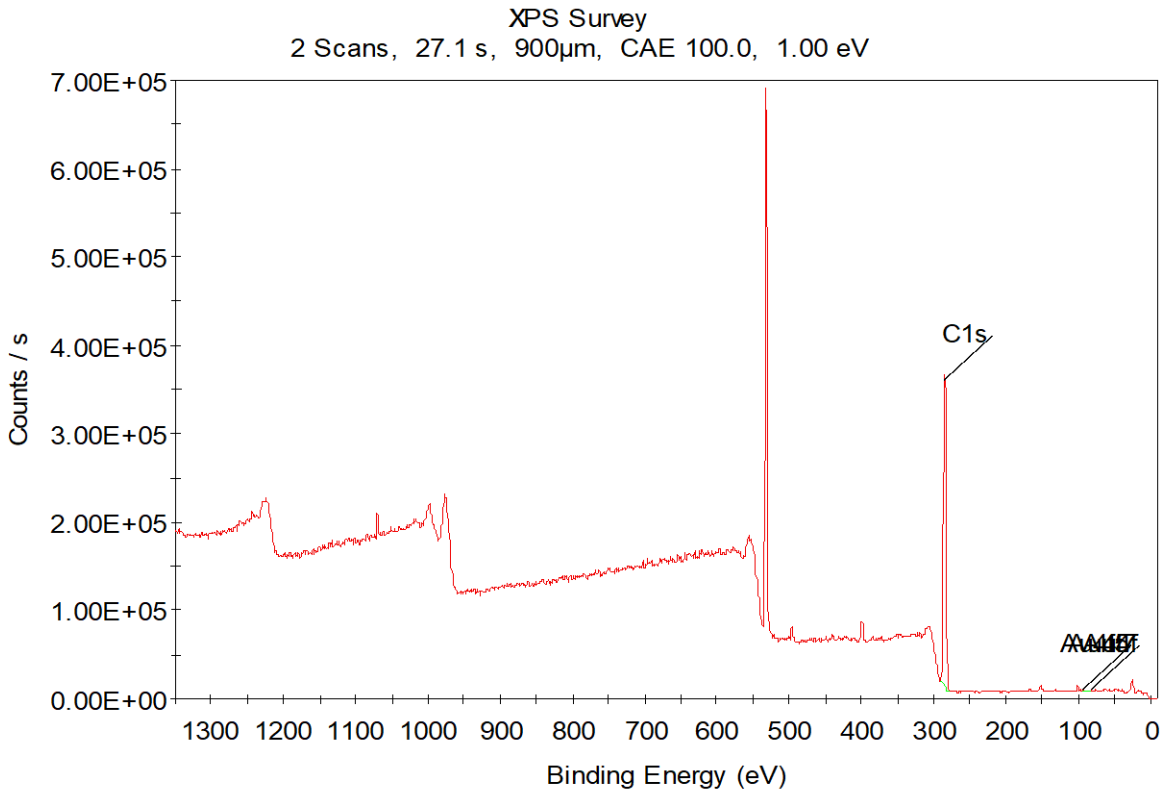


Figure S9. X-ray photoelectron spectroscopy analysis. XPS survey spectrum of progeny bacteria grown after 2nd subculture of Lac_AuNC.

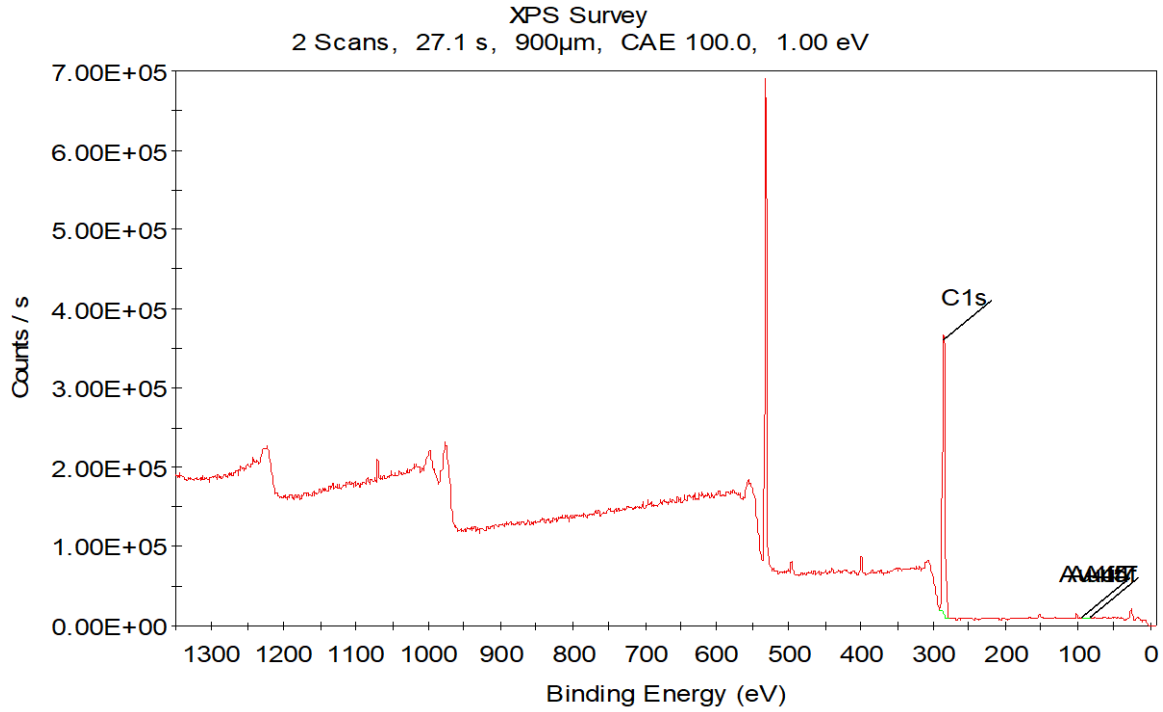


Figure S10. X-ray photoelectron spectroscopy analysis. XPS survey spectrum of progeny bacteria grown after 3rd subculture of Lac_AuNC.

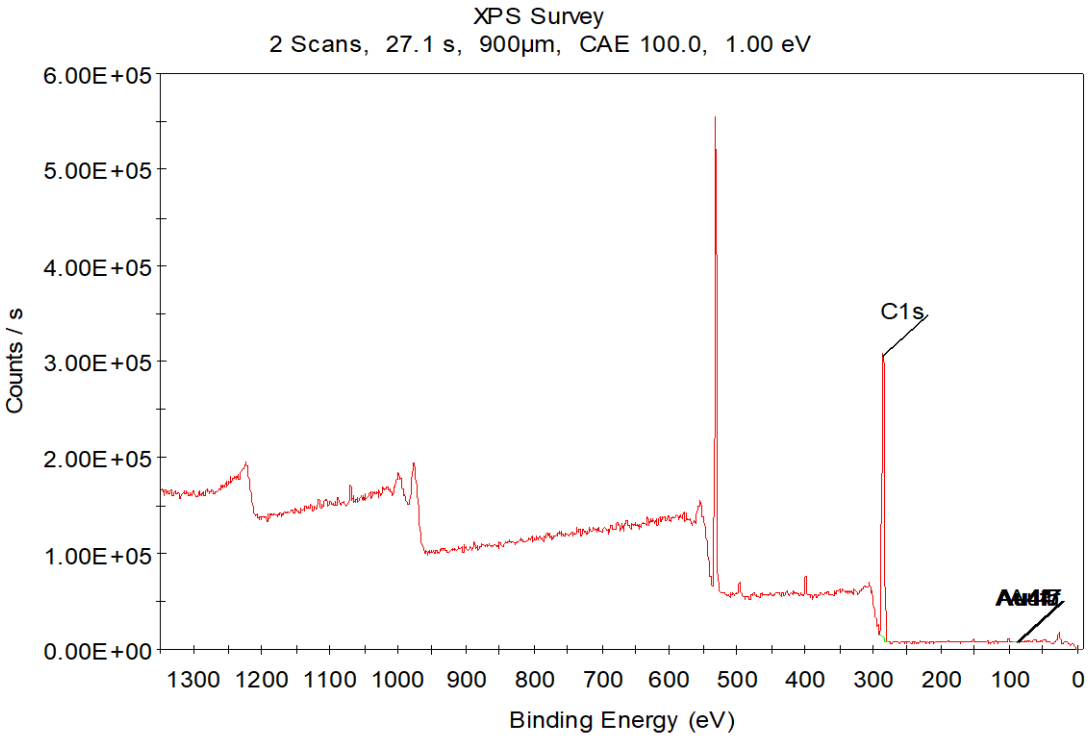


Figure S11. X-ray photoelectron spectroscopy analysis. XPS survey spectrum of progeny bacteria grown after 4th subculture of Lac_AuNC.

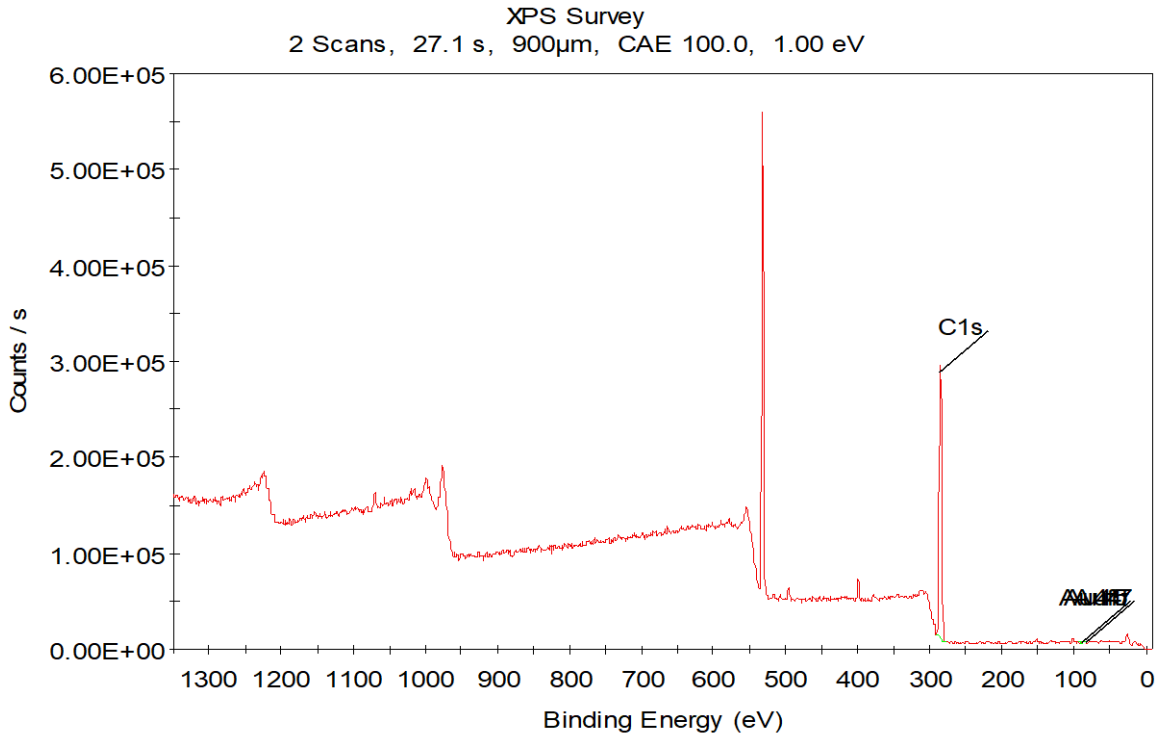


Figure S12. X-ray photoelectron spectroscopy analysis. XPS survey spectrum of progeny bacteria grown after 5th subculture of Lac_AuNC.

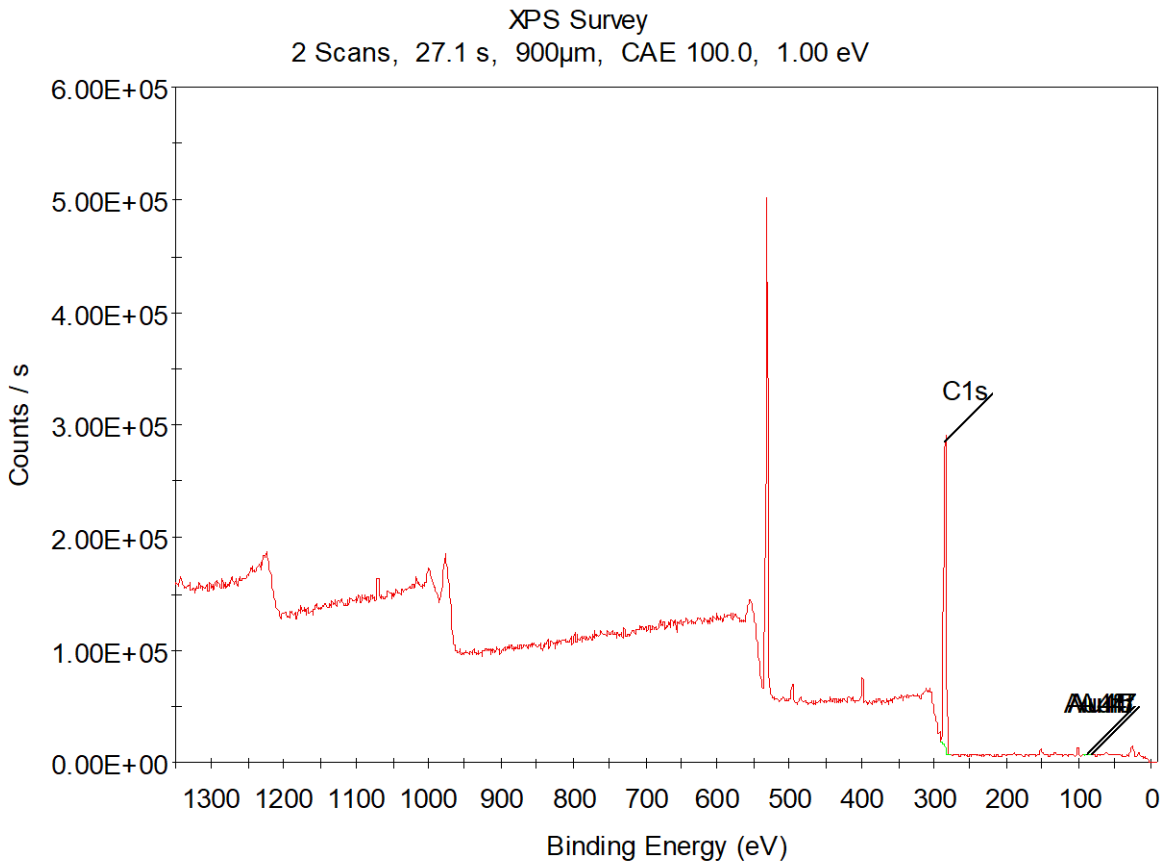
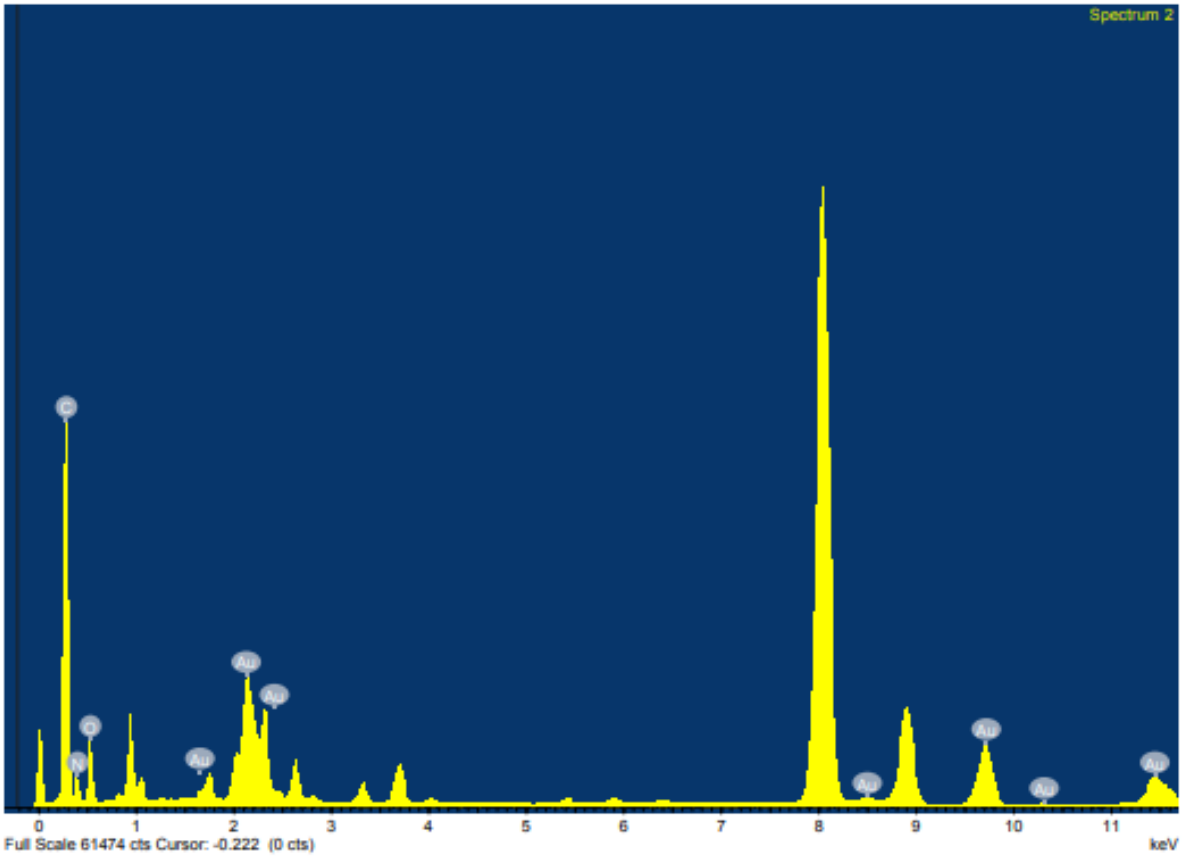


Figure S13. X-ray photoelectron spectroscopy analysis. XPS survey spectrum of progeny bacteria grown after 6th subculture of Lac_AuNC.



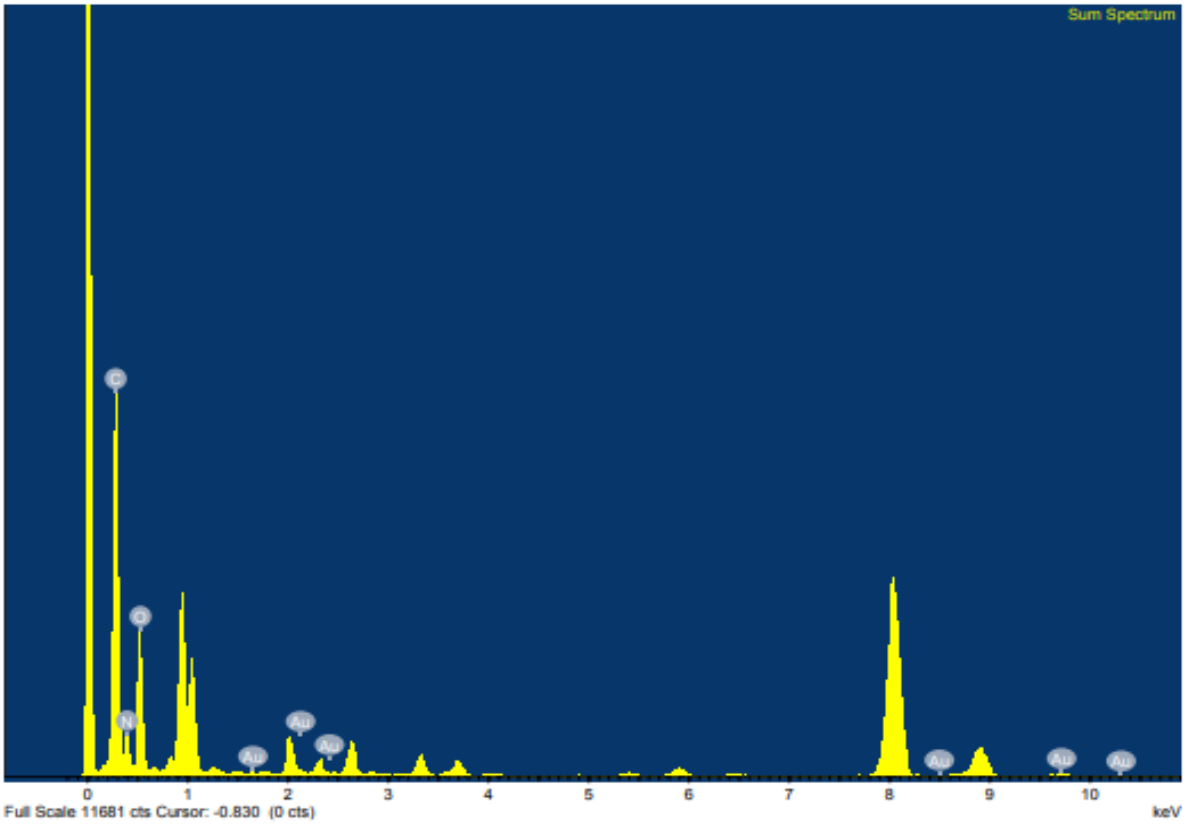
Spectrum processing :
Peaks possibly omitted : 0.680, 0.800, 0.933, 1.057, 1.251, 3.318, 3.693, 4.018, 5.415, 5.895, 6.402, 16.075, 16.914, 17.769 keV

Quantitation method : Cliff Lorimer thin ratio section.
Processing option : All elements analyzed
Number of iterations = 1

Standardless

Element	Weight%	Atomic%
C K	42.82	80.68
N K	3.13	5.06
O K	6.19	8.75
Au L	47.86	5.50
Totals	100.00	

Figure S14. Energy dispersive X-ray analysis. Results of EDX analysis of Lac_AuNC.



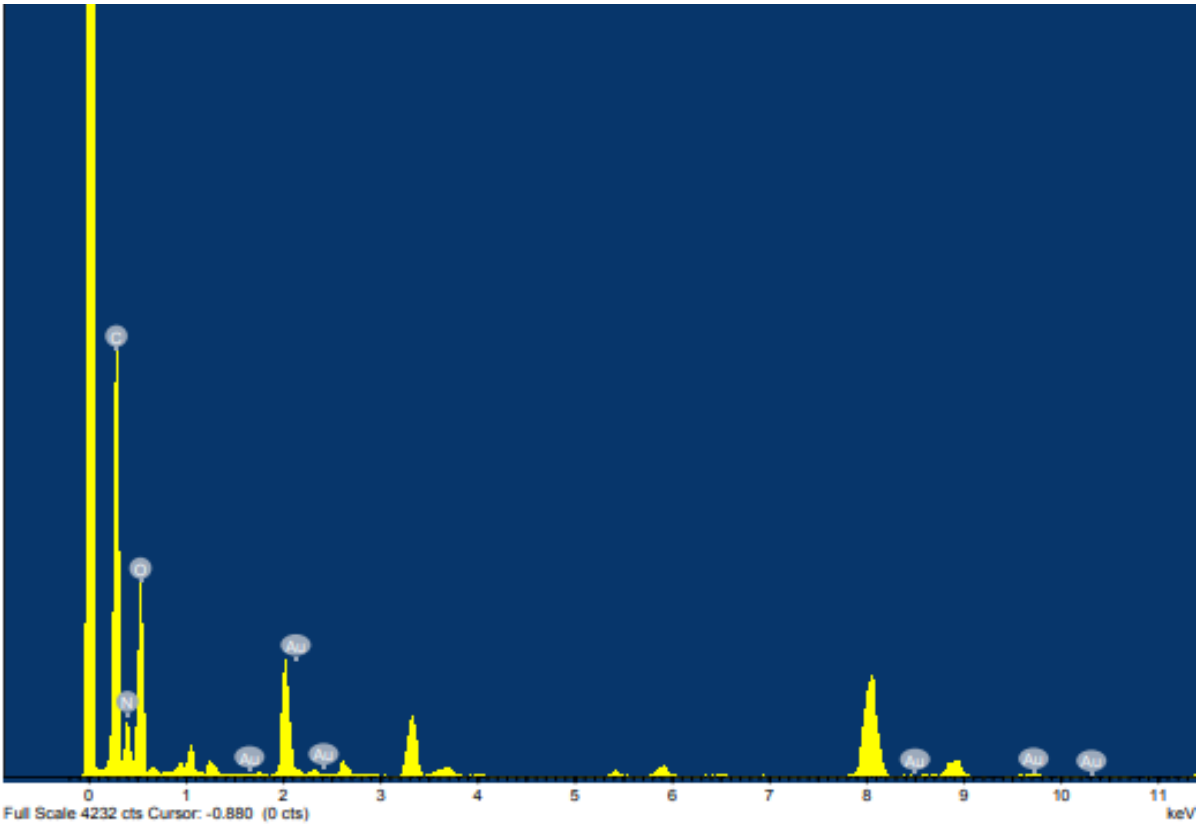
Spectrum processing :
Peaks possibly omitted : 0.660, 0.800, 0.932, 1.050, 1.257, 3.320, 3.696, 3.991, 5.419, 5.894, 6.450 keV

Quantitation method : Cliff Lorimer thin ratio section.
Processing option : All elements analyzed
Number of iterations = 1

Standardless

Element	Weight%	Atomic%
C K	67.99	74.81
N K	7.63	7.20
O K	21.55	17.80
Au L	2.83	0.19
Totals	100.00	

Figure S15. Energy dispersive X-ray analysis. Results of EDX analysis of progeny bacteria grown after 1st subculture of Lac_AuNC.



Full Scale 4232 cts Cursor: -0.880 (0 cts)

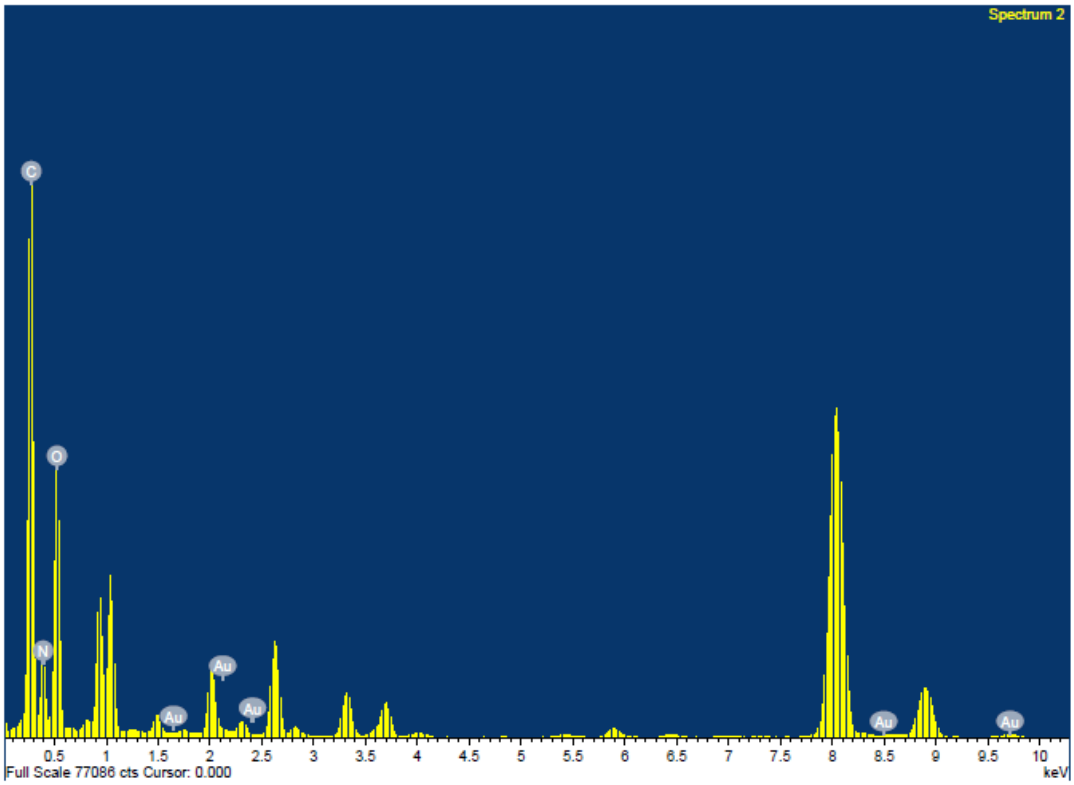
Spectrum processing :
Peaks possibly omitted : 0.660, 0.920, 1.043, 1.246, 3.317, 3.687, 5.408, 5.895, 6.460 keV

Quantitation method : Cliff Lorimer thin ratio section.
Processing option : All elements analyzed
Number of iterations = 1

Standardless

Element	Weight%	Atomic%
C K	64.56	71.35
N K	8.38	7.94
O K	24.77	20.55
Au L	2.30	0.16
Totals	100.00	

Figure S16. Energy dispersive X-ray analysis. Results of EDX analysis of progeny bacteria grown after 2nd subculture of Lac_AuNC.



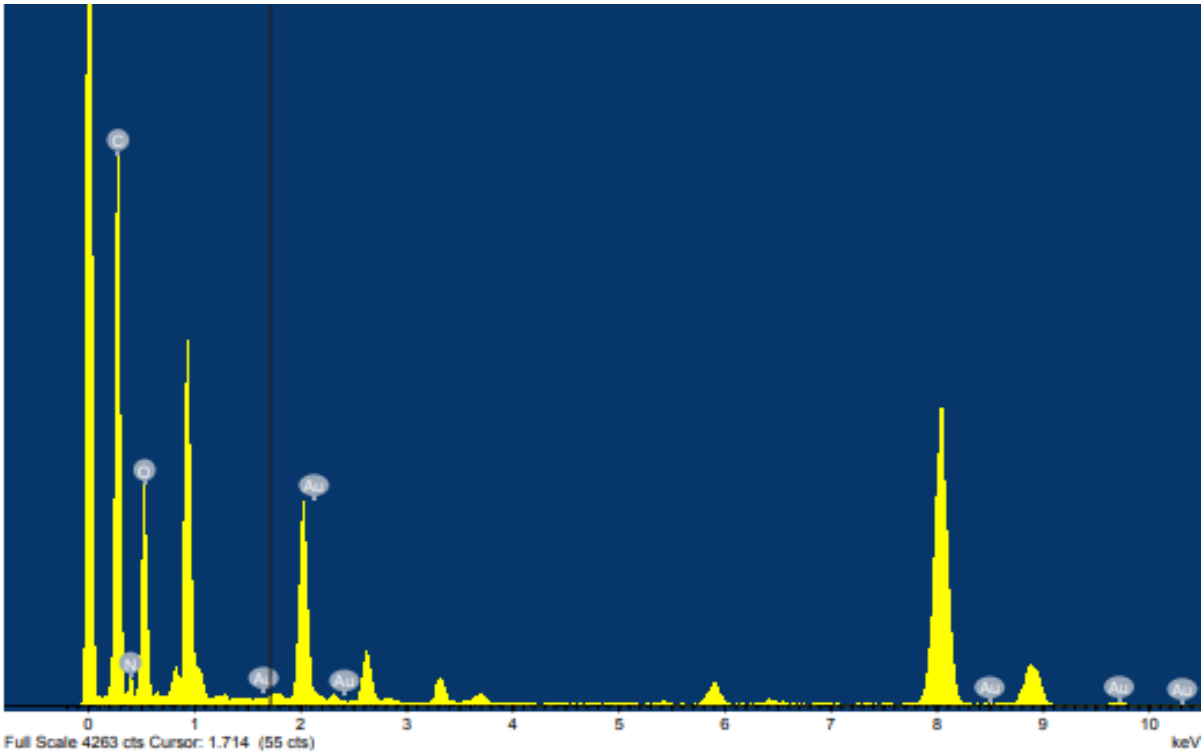
Spectrum processing :
Peaks possibly omitted : 0.660, 0.798, 0.929, 1.048, 1.258, 3.318, 3.693, 4.014, 5.410, 5.896, 6.450, 16.076, 16.930 keV

Quantitation method : Cliff Lorimer thin ratio section.
Processing option : All elements analyzed
Number of iterations = 1

Standardless

Element	Weight%	Atomic%
C K	63.05	69.86
N K	9.02	8.57
O K	25.75	21.42
Au L	2.18	0.15
Totals	100.00	

Figure S17. Energy dispersive X-ray analysis. Results of EDX analysis of progeny bacteria grown after 3rd subculture of Lac_AuNC.



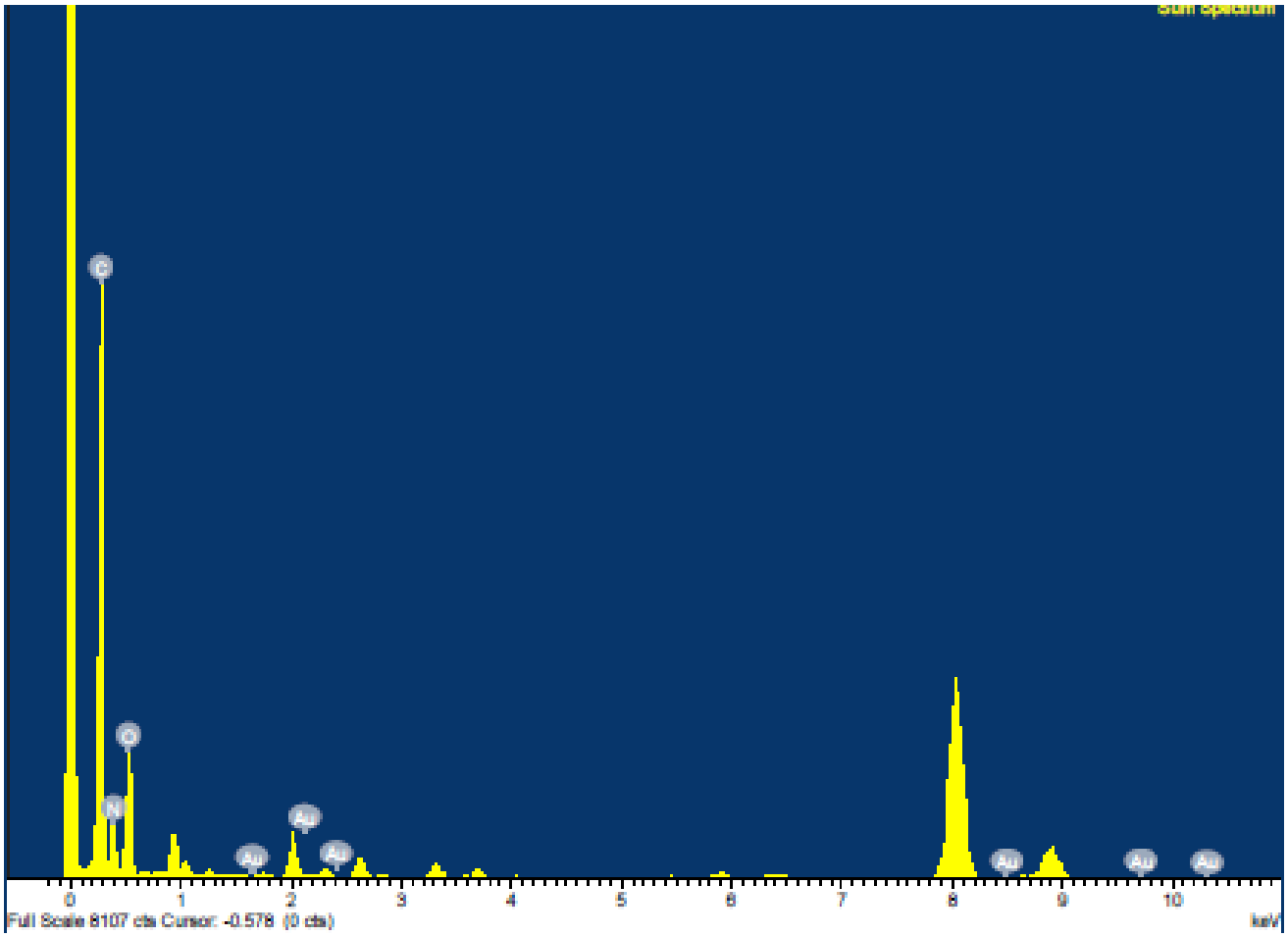
Spectrum processing :
Peaks possibly omitted : 0.800, 0.936, 3.316, 3.692, 5.410, 5.897, 6.432 keV

Quantitation method : Cliff Lorimer thin ratio section.
Processing option : All elements analyzed
Number of iterations = 1

Standardless

Element	Weight%	Atomic%
C K	71.65	78.03
N K	2.64	2.47
O K	23.69	19.37
Au L	2.02	0.13
Totals	100.00	

Figure S18. Energy dispersive X-ray analysis. Results of EDX analysis of progeny bacteria grown after 4th subculture of Lac_AuNC.



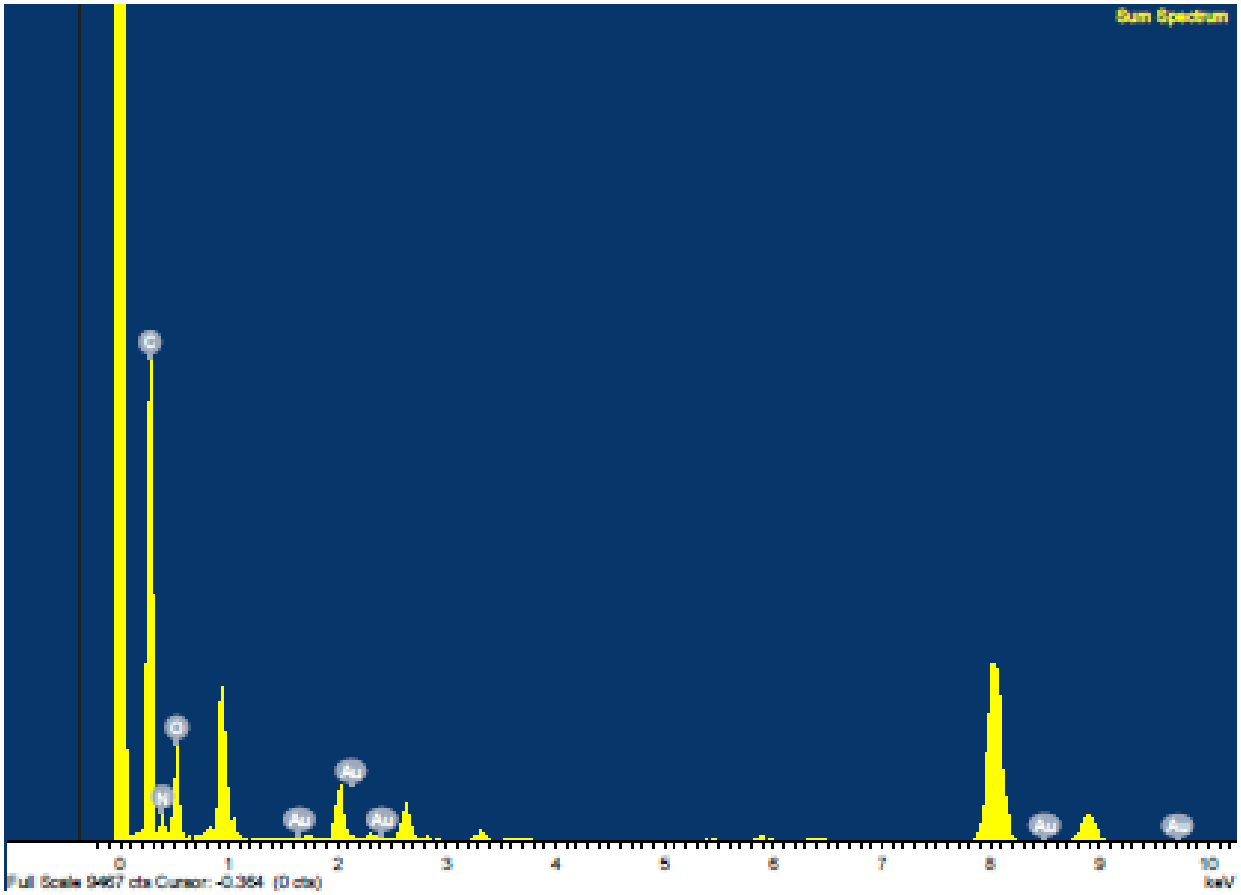
Spectrum processing :
Peaks possibly omitted : 0.932, 1.054, 1.252, 3.315, 3.695, 4.041, 5.430, 5.888, 6.380 keV

Quantitation method : Cliff Lorimer thin ratio section.
Processing option : All elements analyzed
Number of iterations = 1

Standardless

Element	Weight%	Atomic%
C K	76.70	81.55
N K	7.65	6.97
O K	14.26	11.38
Au L	1.39	0.09
Totals	100.00	

Figure S19. Energy dispersive X-ray analysis. Results of EDX analysis of progeny bacteria grown after 5thsubculture of Lac_AuNC.



Spectrum processing :
Peaks possibly omitted : 0.800, 0.934, 3.319, 3.700, 5.410, 5.888, 6.421 keV

Quantitation method : Cliff Lorimer thin ratio section.
Processing option : All elements analyzed
Number of iterations = 1

Standardless

Element	Weight%	Atomic%
C K	80.20	84.60
N K	4.88	4.42
O K	13.78	10.91
Au L	1.13	0.07
Totals	100.00	

Figure S20. Energy Dispersive X-ray analysis. Results of EDX analysis of progeny bacteria grown after 6thsubculture of Lac_AuNC.

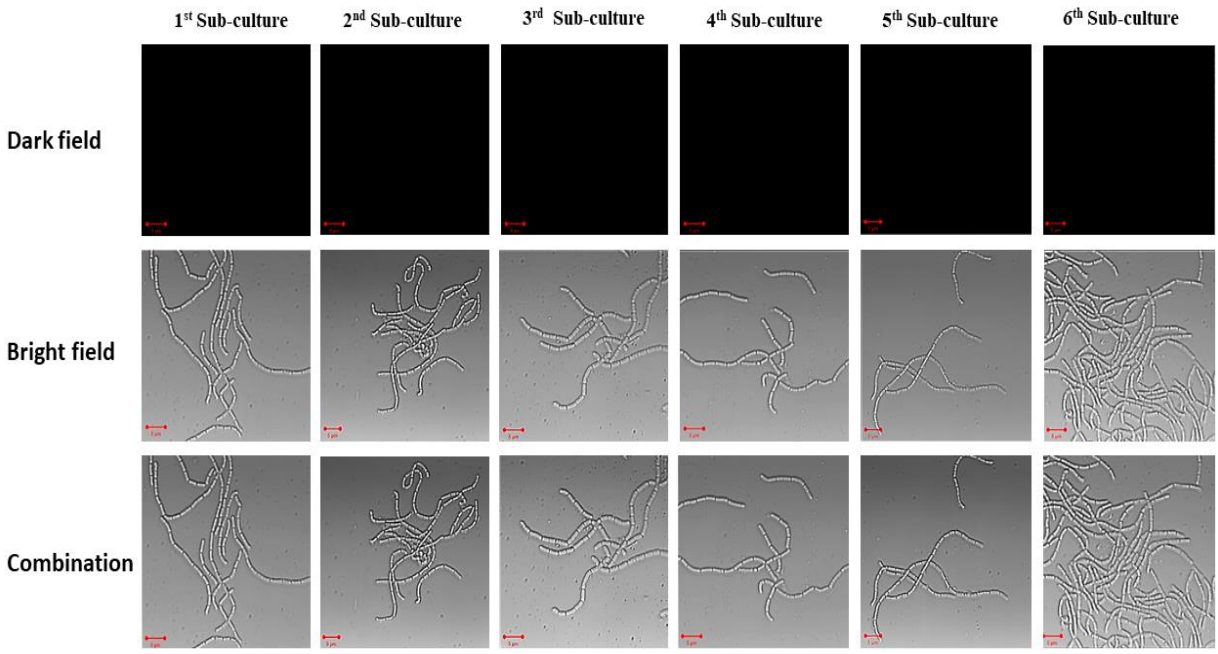


Figure S21. Confocal laser scanning microscopy. CLSM images of progeny bacteria grown from 1st to 6th subculture of Lac_AuNC as described in the legends.

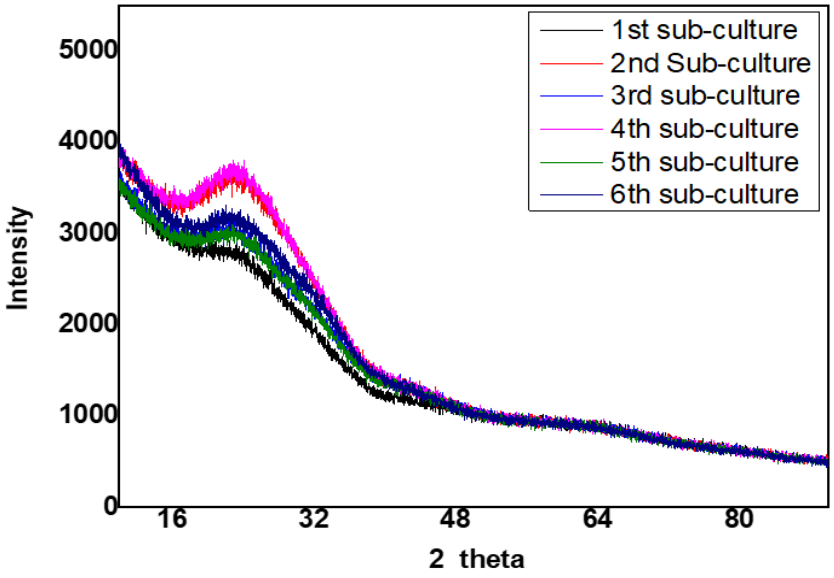


Figure S22. X-ray diffraction analysis. X-ray diffraction pattern of progeny bacteria grown from 1st to 6th subculture of Lac_AuNC as described in the legends.

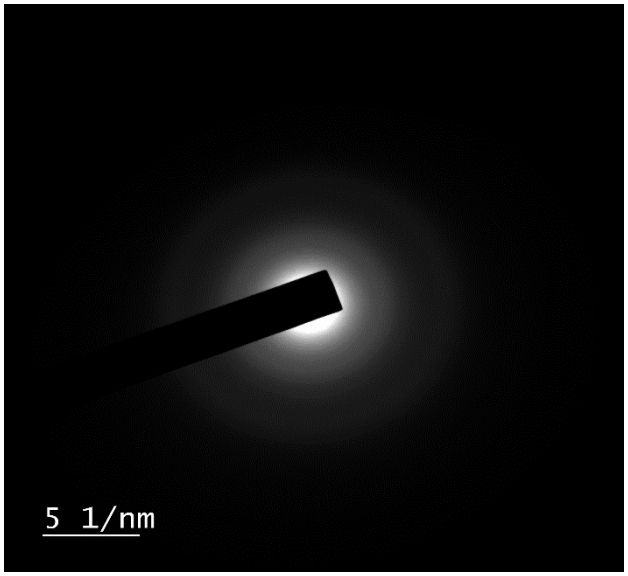


Figure S23. Selected Area Electron Diffraction analysis. SAED pattern of Lac_AuNC.

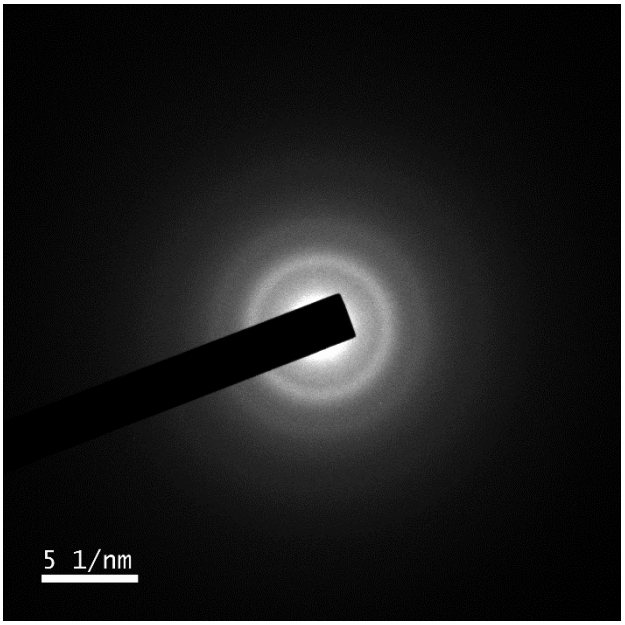


Figure S24. Selected Area Electron Diffraction analysis. SAED pattern of 1st subculture progeny of Lac_AuNC.

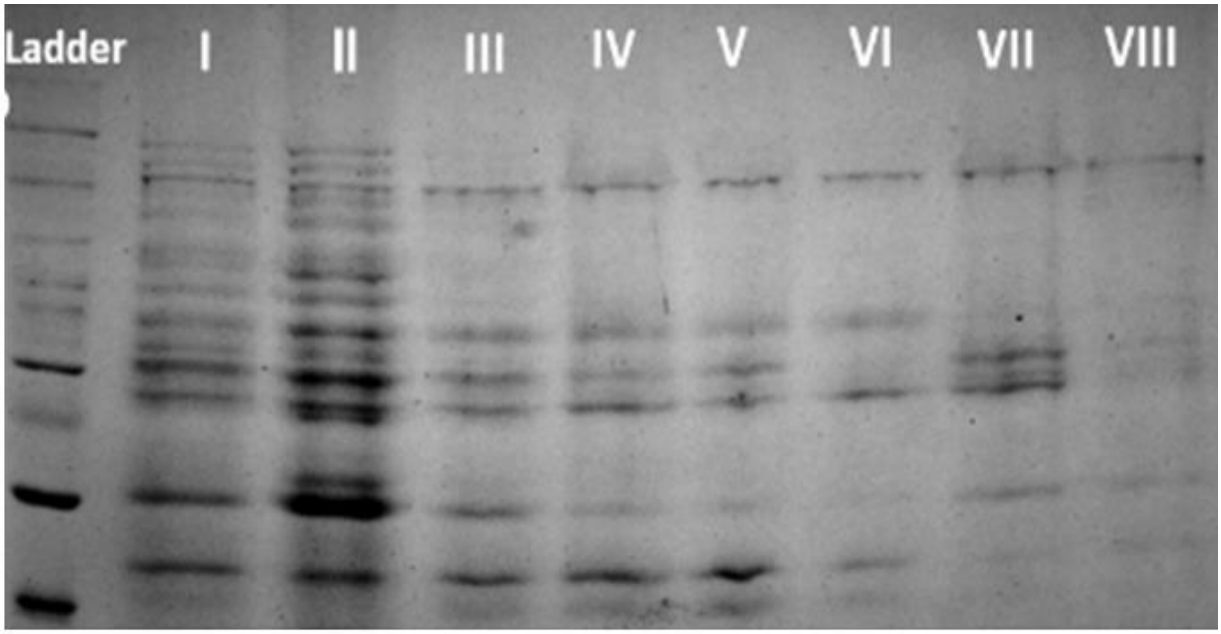


Figure S25. Total protein isolation bands of (I) Lac_AuNC, (II) control *L.rhamnosus*, (III)1st subculture, (IV) 2ndsubculture, (V) 3rdsubculture, (VI) 4th subculture, (VII) 5th subculture, and (VIII) 6th subculture.

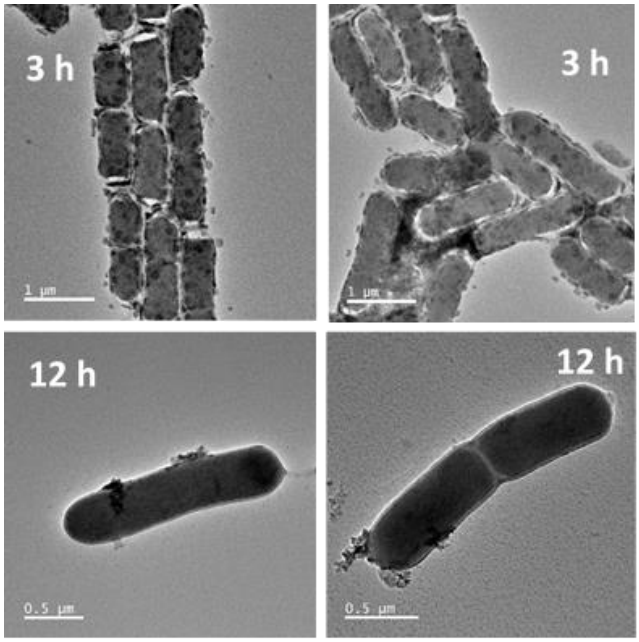


Figure S26. FETEM images of progenies of Lac_AuNC collected after 3 h and 12 h of incubation.

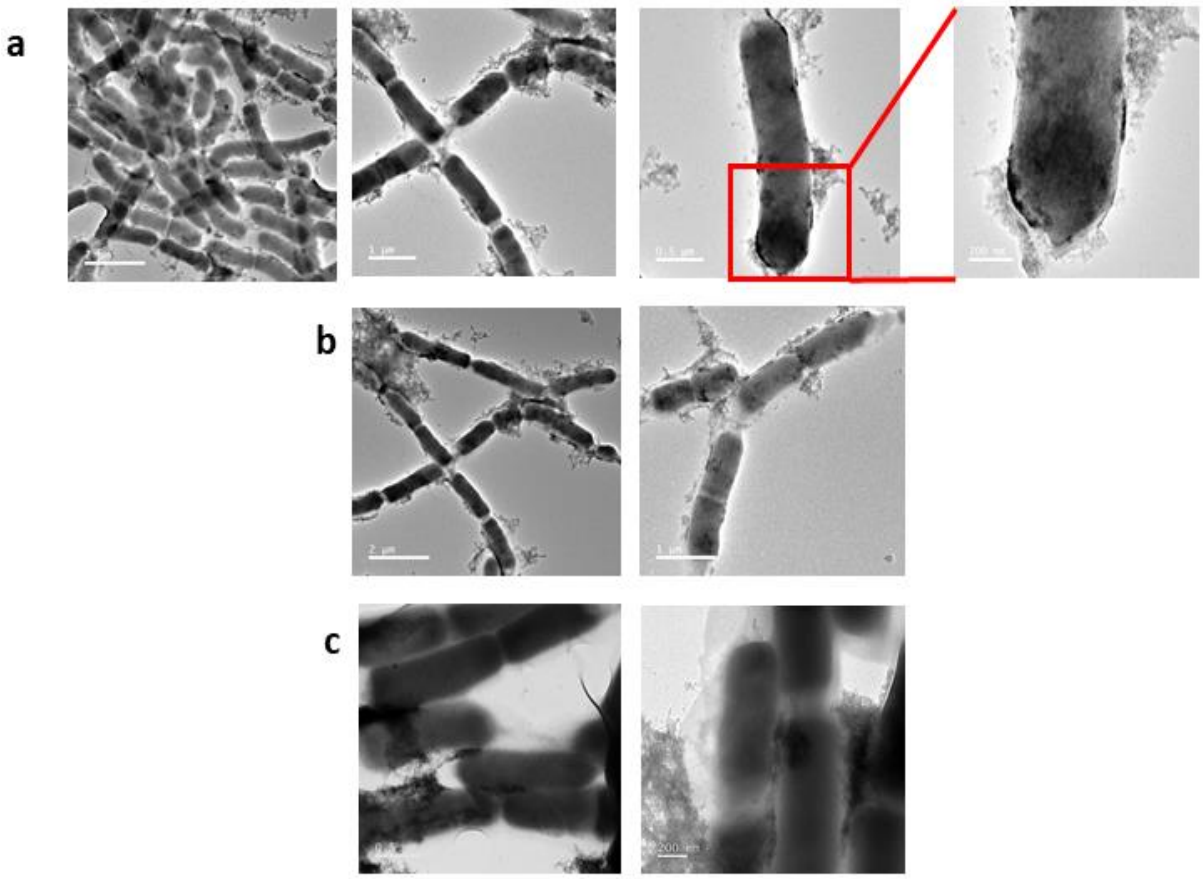


Figure S27. (a) FETEM images of Lac_AuNC taken on the same day as provided in Figure 2 of manuscript (b) and (c) FETEM images of Lac_AuNC (sample prepared and image taken on days other than that mentioned in the manuscripts).