



Characterization of SeNPs produced by *B. mycoides* SelTE01 and *S.* *maltophilia* SelTE02 and their biomolecular organic material

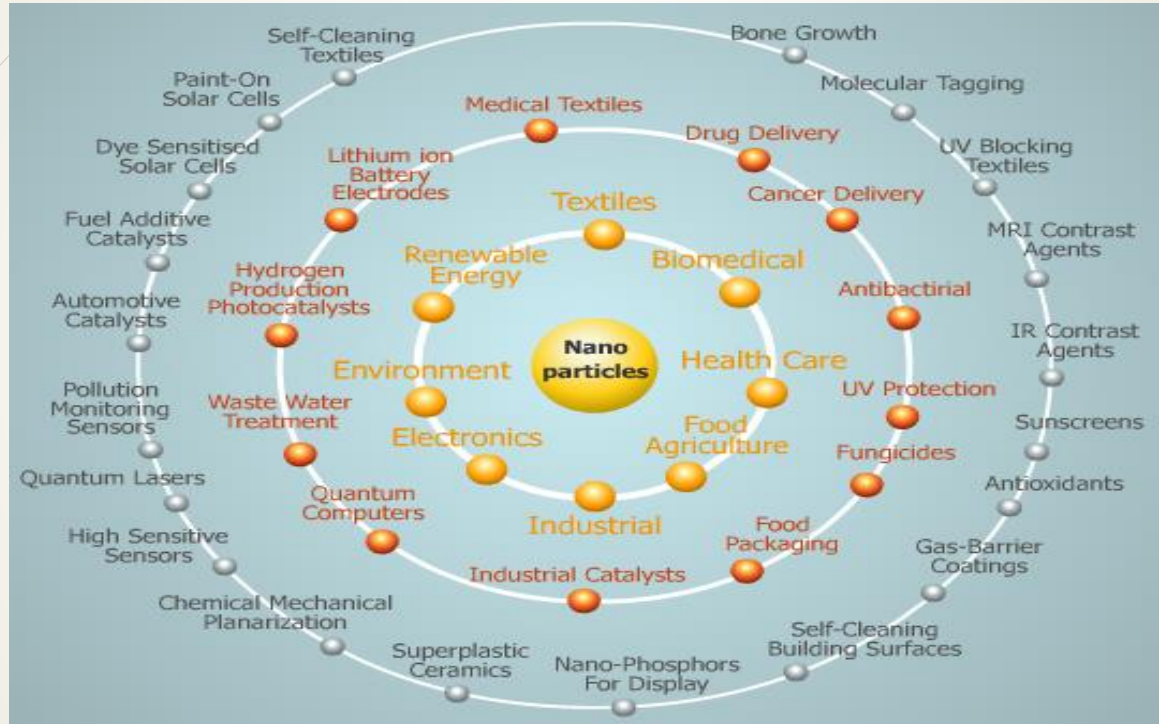
Presenting author: **Elena Piacenza**

September 23, 2016



Introduction

Application fields of nanoparticles



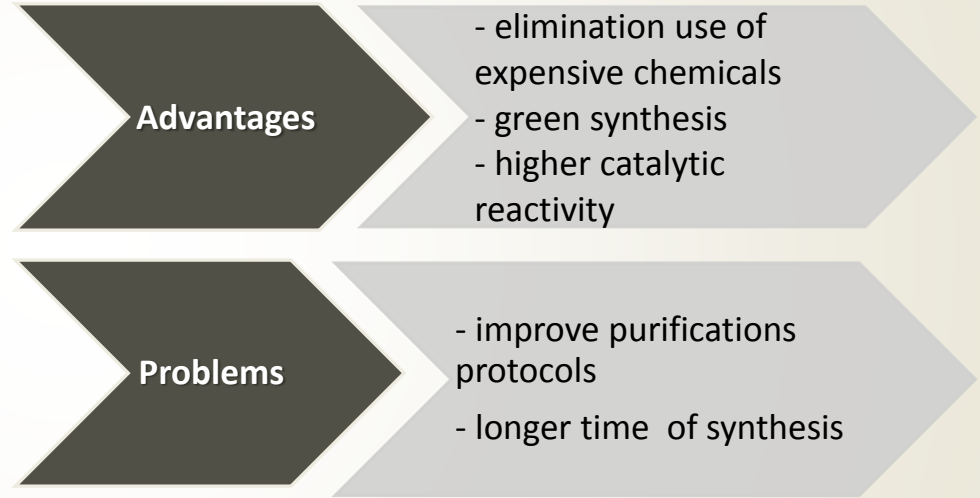
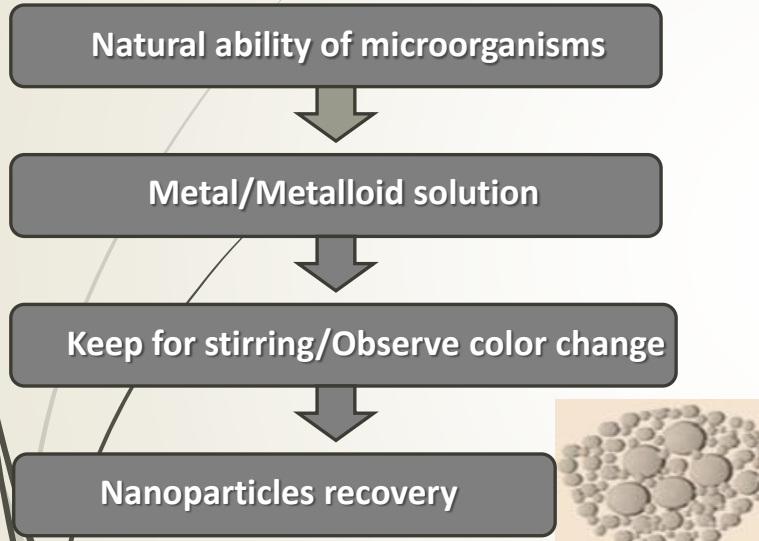
Methods of nanostructures synthesis

- high costs
- hazardous toxic wastes
- use of toxic chemicals

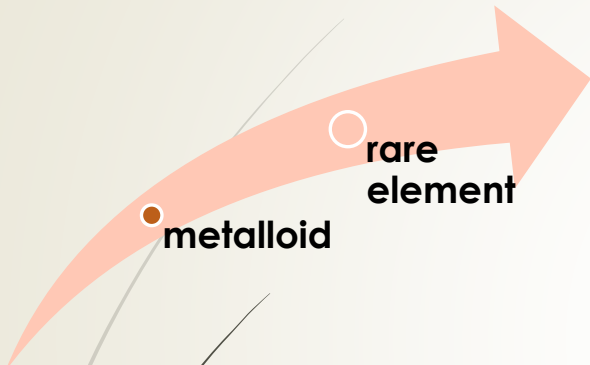
Biological methods

Biogenic nanoparticles

Biogenic nanoparticles



The Chalcogen Selenium



Periodic Table of the Elements

| | | | | | | | | | | | | | | | | | |
|--------------------------------|---------------------------------|--------------------------------|-----------------------------------|---------------------------------|-------------------------------------|----------------------------------|-----------------------------------|----------------------------------|----------------------------------|----------------------------------|----------------------------------|------------------------------------|------------------------------------|---------------------------------|-------------------------------------|----------------------------------|------------------------------------|
| 1 1IA 11A | 2 IIA 2A | | | | | | | | | | | 13 IIIA 3A | 14 IVA 4A | 15 VA 5A | 16 VIA 6A | 17 VIIA 7A | 18 VIIIA 8A |
| 1 H Hydrogen 1.008 | 2 He Helium 4.003 | | | | | | | | | | | 3 B Boron 10.811 | 4 C Carbon 12.011 | 5 N Nitrogen 14.007 | 6 O Oxygen 15.999 | 7 F Fluorine 18.998 | 8 Ne Neon 20.180 |
| 3 Li Lithium 6.941 | 4 Be Beryllium 9.012 | | | | | | | | | | | 9 Al Aluminum 26.982 | 10 Si Silicon 28.086 | 11 P Phosphorus 30.974 | 12 S Sulfur 32.06 | 13 Cl Chlorine 35.45 | 14 Ar Argon 39.948 |
| 11 Na Sodium 22.990 | 12 Mg Magnesium 24.305 | 3 Sc Scandium 44.956 | 4 Ti Titanium 47.88 | 5 V Vanadium 50.942 | 6 Cr Chromium 51.996 | 7 Mn Manganese 54.938 | 8 Fe Iron 55.845 | 9 Co Cobalt 58.933 | 10 Ni Nickel 58.693 | 11 Cu Copper 63.546 | 12 Zn Zinc 65.38 | 13 Ga Gallium 69.723 | 14 Ge Germanium 72.63 | 15 As Arsenic 74.922 | 16 Se Selenium 78.96 | 17 Br Bromine 79.904 | 18 Kr Krypton 83.80 |
| 19 K Potassium 39.098 | 20 Ca Calcium 40.078 | 21 Sc Scandium 44.956 | 22 Ti Titanium 47.88 | 23 V Vanadium 50.942 | 24 Cr Chromium 51.996 | 25 Mn Manganese 54.938 | 26 Fe Iron 55.845 | 27 Co Cobalt 58.933 | 28 Ni Nickel 58.693 | 29 Cu Copper 63.546 | 30 Zn Zinc 65.38 | 31 Ga Gallium 69.723 | 32 Ge Germanium 72.63 | 33 As Arsenic 74.922 | 34 Se Selenium 78.96 | 35 Br Bromine 79.904 | 36 Kr Krypton 83.80 |
| 37 Rb Rubidium 85.468 | 38 Sr Strontium 87.62 | 39 Y Yttrium 88.906 | 40 Zr Zirconium 91.224 | 41 Nb Niobium 92.906 | 42 Mo Molybdenum 95.94 | 43 Tc Technetium 98.906 | 44 Ru Ruthenium 101.07 | 45 Rh Rhodium 102.905 | 46 Pd Palladium 106.367 | 47 Ag Silver 107.868 | 48 Cd Cadmium 112.411 | 49 In Indium 114.818 | 50 Sn Tin 118.710 | 51 Sb Antimony 121.757 | 52 Te Tellurium 127.6 | 53 I Iodine 126.905 | 54 Xe Xenon 131.29 |
| 55 Cs Cesium 132.905 | 56 Ba Barium 137.327 | 57-71 Lanthanide Series | 72 Hf Hafnium 178.49 | 73 Ta Tantalum 180.948 | 74 W Tungsten 183.84 | 75 Re Rhenium 186.207 | 76 Os Osmium 190.23 | 77 Ir Iridium 192.22 | 78 Pt Platinum 195.084 | 79 Au Gold 196.967 | 80 Hg Mercury 200.59 | 81 Tl Thallium 204.384 | 82 Pb Lead 207.2 | 83 Bi Bismuth 208.980 | 84 Po Polonium 209 | 85 At Astatine 210 | 86 Rn Radon 222 |
| 87 Fr Francium 223 | 88 Ra Radium 226 | 89-103 Actinide Series | 104 Rf Rutherfordium 261 | 105 Db Dubnium 262 | 106 Sg Seaborgium 266 | 107 Bh Bohrium 264 | 108 Hs Hassium 277 | 109 Mt Meitnerium 268 | 110 Ds Darmstadtium 285 | 111 Rg Roentgenium 282 | 112 Cn Copernicium 285 | 113 Nh Nihonium 284 | 114 Fl Flerovium 289 | 115 Uu Ununpentium 288 | 116 Uuh Ununhexium 289 | 117 Uus Ununseptium 289 | 118 Uuo Ununoctium 294 |
| Lanthanide Series | | | 57 La Lanthanum 138.905 | 58 Ce Cerium 140.12 | 59 Pr Praseodymium 140.908 | 60 Nd Neodymium 144.24 | 61 Pm Promethium 144.913 | 62 Sm Samarium 150.36 | 63 Eu Europium 151.964 | 64 Gd Gadolinium 157.25 | 65 Tb Terbium 158.925 | 66 Dy Dysprosium 162.50 | 67 Ho Holmium 164.930 | 68 Er Erbium 167.259 | 69 Tm Thulium 168.930 | 70 Yb Ytterbium 173.054 | 71 Lu Lutetium 174.967 |
| Actinide Series | | | 89 Ac Actinium 227 | 90 Th Thorium 232.038 | 91 Pa Protactinium 231.036 | 92 U Uranium 238.029 | 93 Np Neptunium 237.048 | 94 Pu Plutonium 244.064 | 95 Am Americium 243.061 | 96 Cm Curium 247.070 | 97 Bk Berkelium 247.070 | 98 Cf Californium 251.083 | 99 Es Einsteinium 252.083 | 100 Fm Fermium 257.103 | 101 Md Mendelevium 258.103 | 102 No Nobelium 259.103 | 103 Lr Lawrencium 260.103 |

Adapted from <http://sciencenotes.org/periodic-table-wallpaper-2/>

Selenium is an essential micronutrient for humans

present in active site of important enzymes

Uses of Se

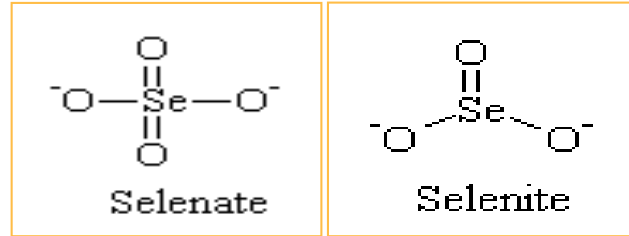
- electronics and glass industry
- animal feeds and food supplements
- photocopying
- metal alloys for batteries
- pigments and ceramics
- plastics and lubricants

Selenium in environment

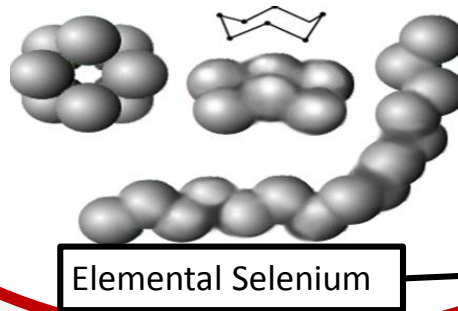
4 valence states :

- Selenite (Se^{4+})
- Selenate (Se^{6+})
- Selenide (Se^{2-})
- Elemental selenium (Se^0)

Most toxic forms



Less toxic form



Selenium nanoparticles (SeNPs)

Production of SeNPs using bacterial strains as detoxification process



Bacillus mycoides SelTE01
Stenotrophomonas maltophilia SelTE02



Minimal Inhibitory
Concentration (MIC) at
25 and 50 mM of
 Na_2SeO_3



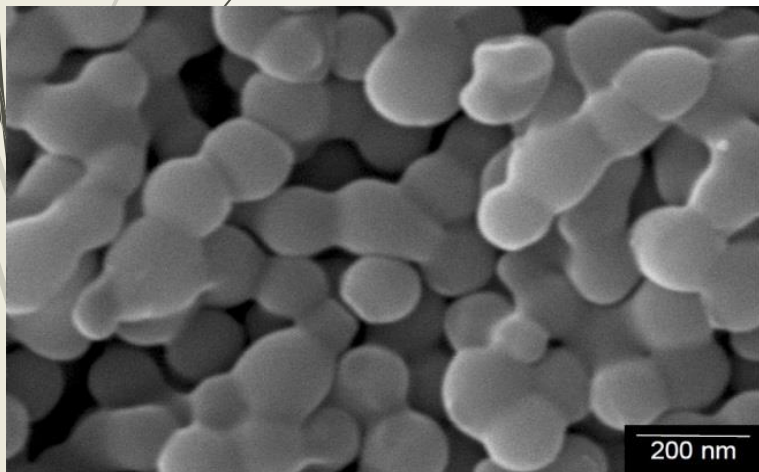
SeNPs
production

SeNPs produced by *B. mycoides* SelTE01 after 24 h of Na₂SeO₃ exposure

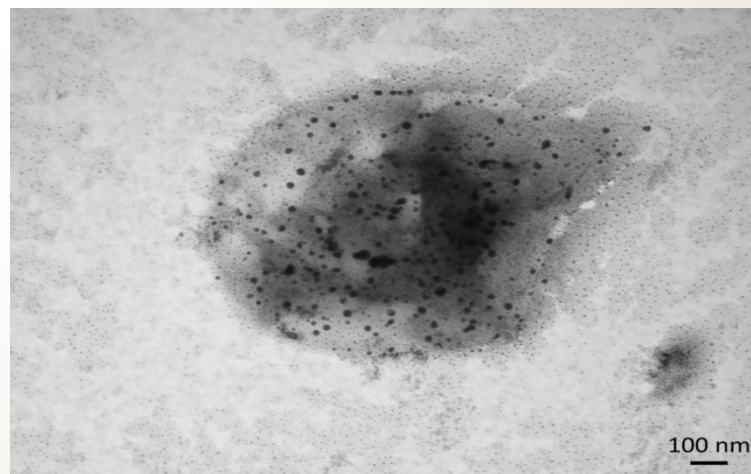
Dynamic Light Scattering (DLS) and Zeta potential measurements

| Size (nm) | Intensity Distribution Data (%) | Std. Dev. | Polydispersity Index (PDI) | Zeta pot. (mV) | Std. Dev. |
|-----------|---------------------------------|-----------|----------------------------|----------------|-----------|
| 142 | 64.35 | 15.78 | 0.21 | -24 | 0.92 |

Scanning Electron Microscopy (SEM)



Transmission Electron Microscopy (TEM)

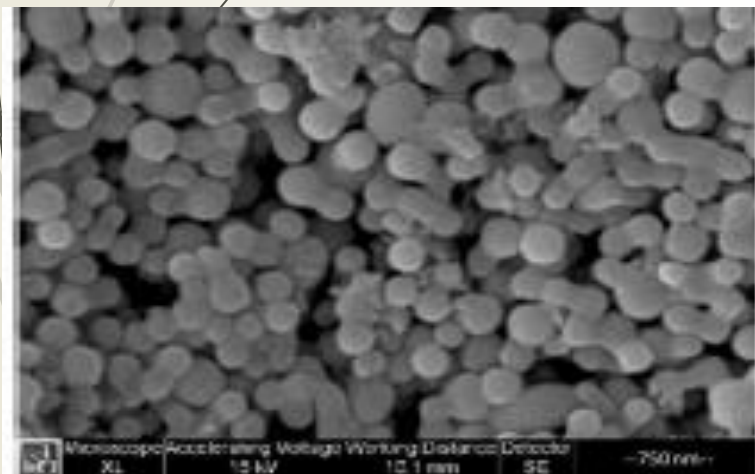


SeNPs produced by *S. maltophilia* SelTE02 after 24 h of Na_2SeO_3 exposure

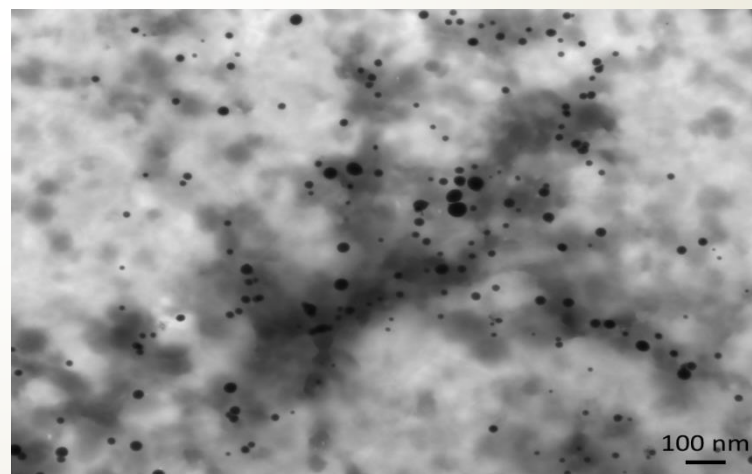
Dynamic Light Scattering (DLS) and Zeta potential measurements

| Size (nm) | Intensity Distribution Data (%) | Std. Dev. | Polydispersity Index (PDI) | Zeta pot. (mV) | Std. Dev. |
|-----------|---------------------------------|-----------|----------------------------|----------------|-----------|
| 396 | 20.75 | 3.73 | 0.22 | -28 | 1.42 |

Scanning Electron Microscopy (SEM)

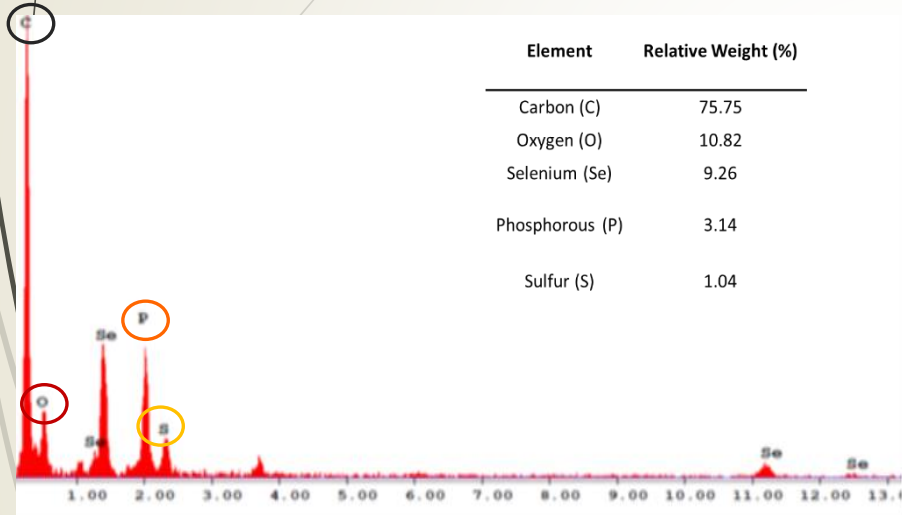


Transmission Electron Microscopy (TEM)

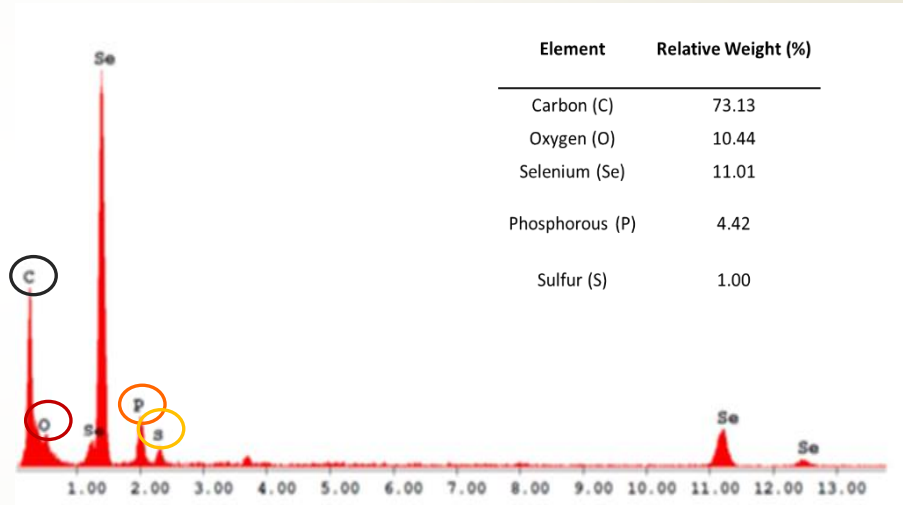


Energy Dispersed X-Ray Spectroscopy (EDX) analysis

SeNPs produced by *B. mycoides* SeTE01



SeNPs produced by *S. maltophilia* SeTE02



Presence of **Carbon**, **Oxygen**, **Phosphorous** and **Sulfur** and TEM images suggest the evidence of biomolecular organic material surrounding SeNPs

Biomolecular organic material surrounding SeNPs

Modifications of the standard extraction protocol to understand the role of the organic surrounding material

Re-exposure of the same biomass to fresh medium with new Na_2SeO_3 (conditioned cells)

Solubilization in 1.5 M Tris-HCl

Sonication

Octanol Fractioning (Over Night)

3 step of octanol fractioning non Over Night

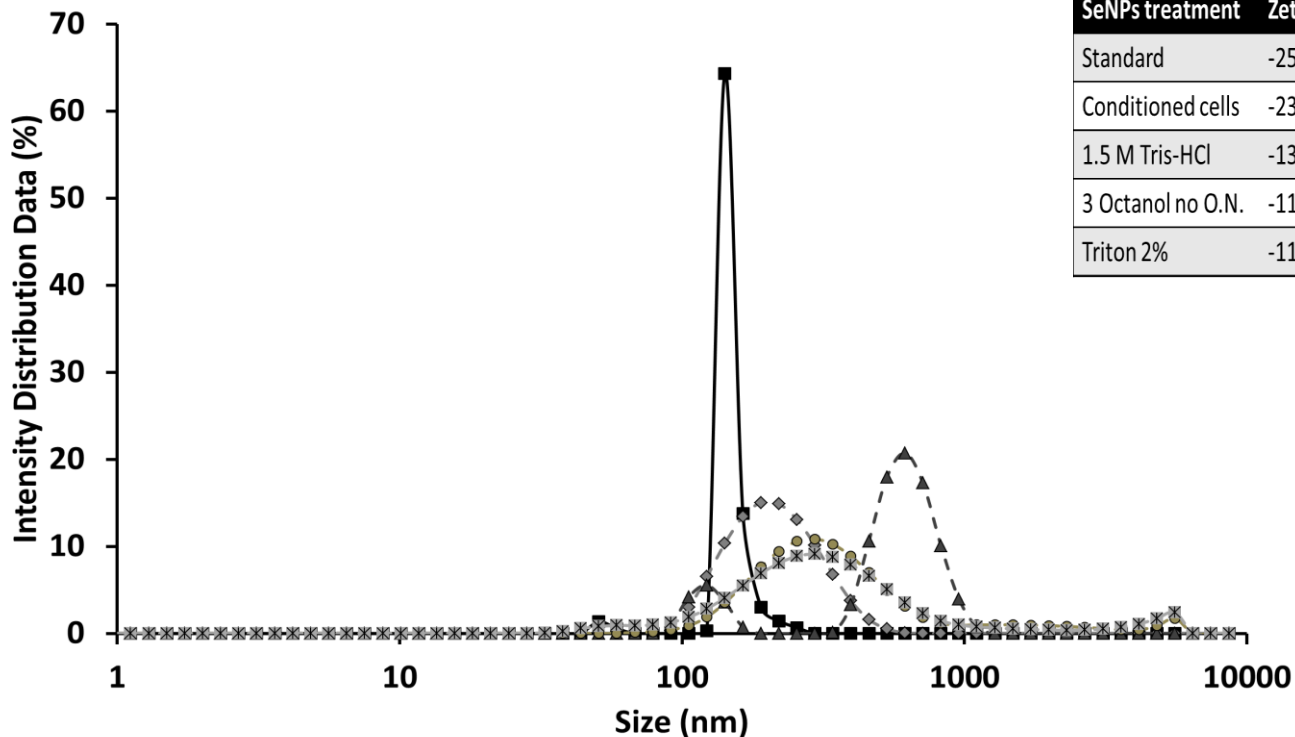
SeNPs recovery

Treatment with 2% Triton X-100

Chemical-physical characterization of treated or modified biogenic SeNPs

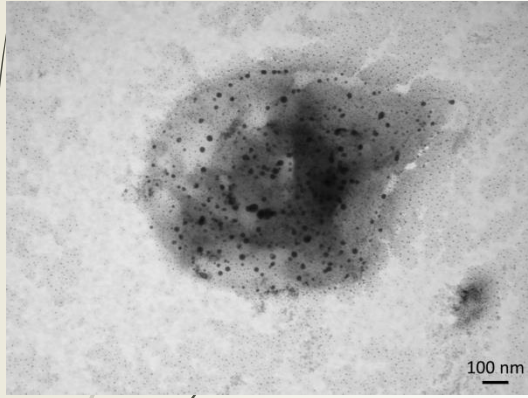
SeNPs produced by *B. mycoides* SeITE01

Zeta potential analysis

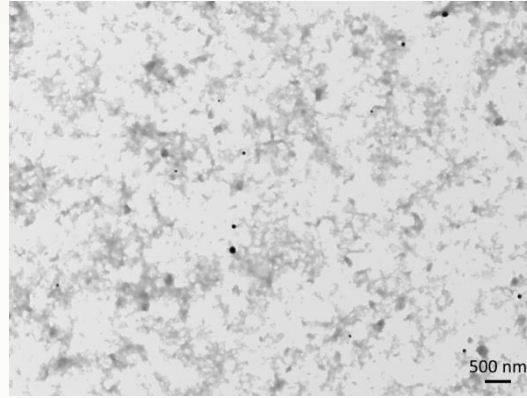


TEM imaging

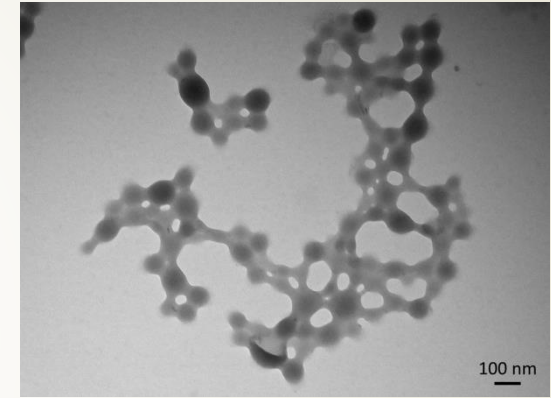
SeNPs standard



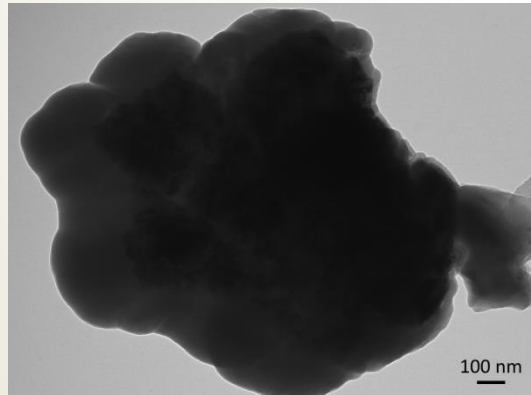
SeNPs from conditioned cells



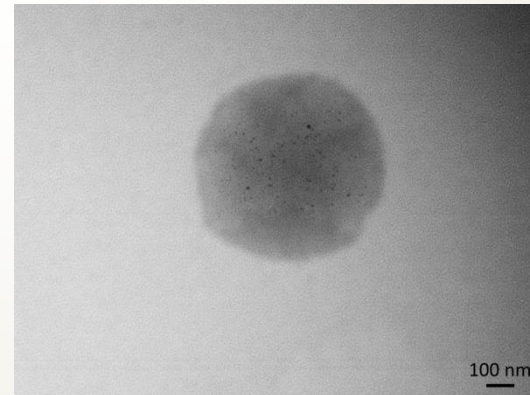
SeNPs with 1.5 M Tris-HCl



SeNPs 3 oct. no O.N.

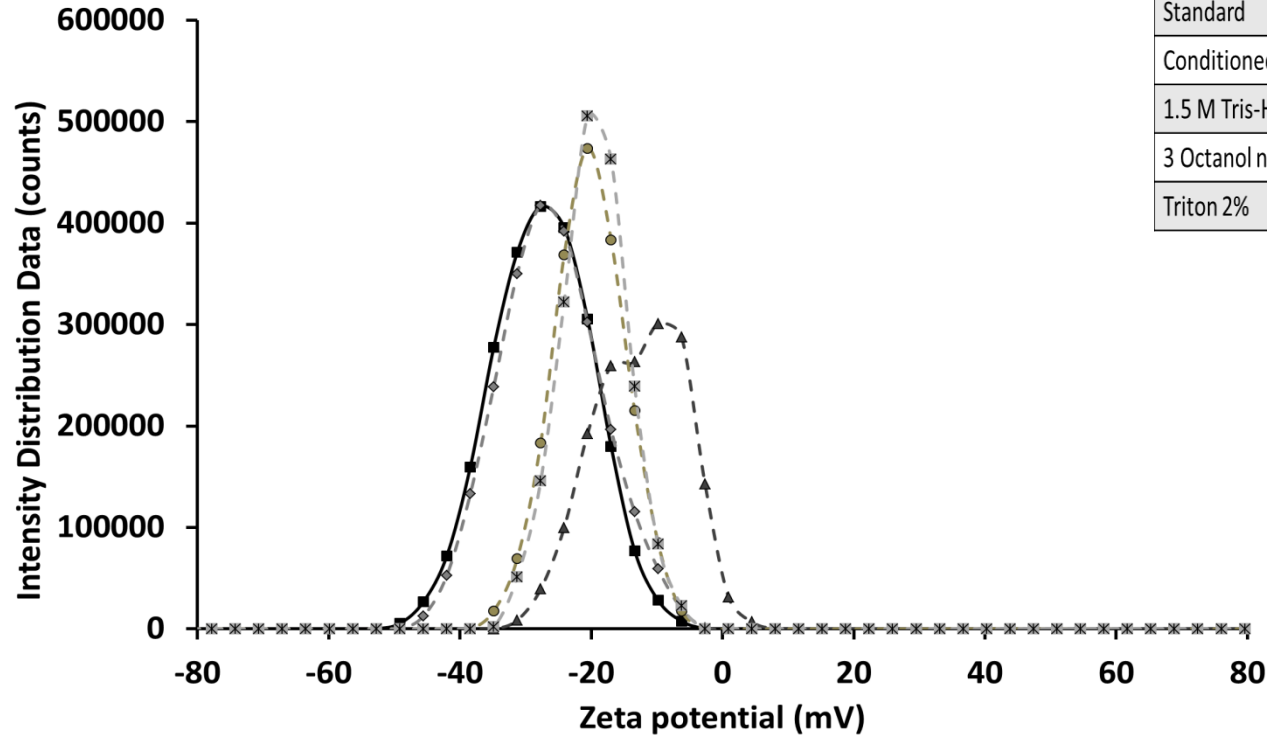


SeNPs with Triton 2%



SeNPs produced by *S. maltophilia* SeITE02

Zeta potential analysis

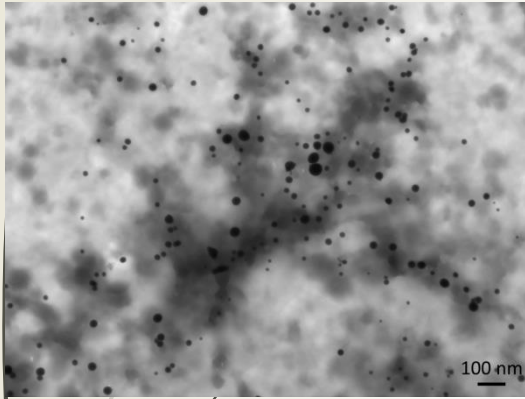


| SeNPs treatment | Zeta potential (mV) |
|-------------------|---------------------|
| Standard | -28 |
| Conditioned cells | -27 |
| 1.5 M Tris-HCl | -7 |
| 3 Octanol no O.N. | -19 |
| Triton 2% | -17 |

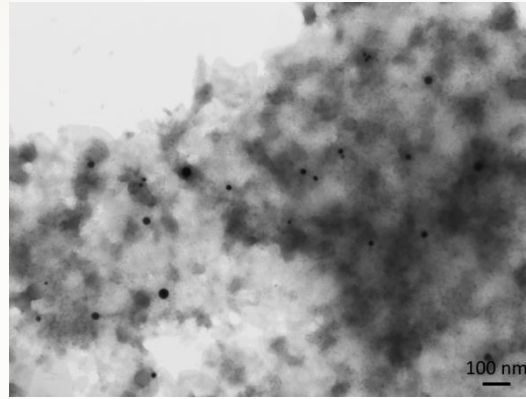
- standard
- ◆-conditioned cells
- ▲-1.5 M Tris-HCl
- 3 oct. No O.N.
- ✖-Triton 2%

TEM imaging

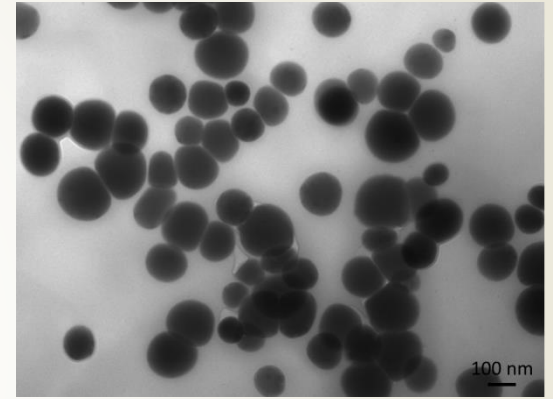
SeNPs standard



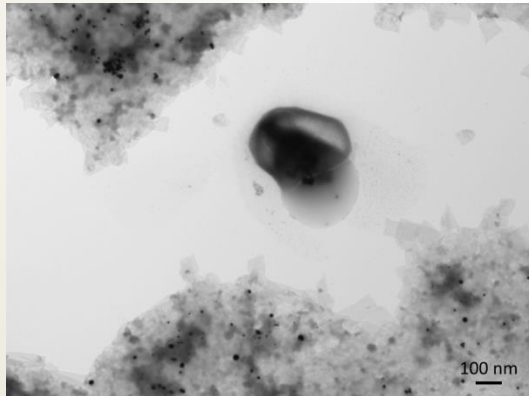
SeNPs from conditioned cells



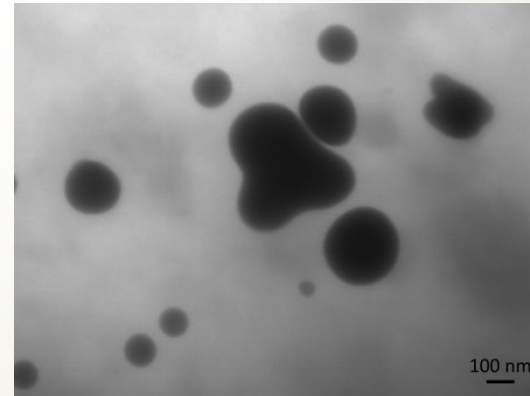
SeNPs with 1.5 M Tris-HCl



SeNPs 3 oct. no O.N.



SeNPs with Triton 2%

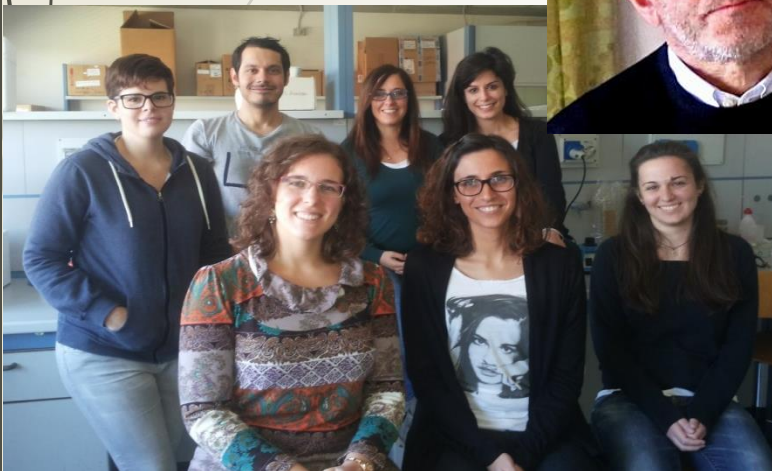


Summary

- ▶ SeNPs produced by both *B. mycoides* SelTE01 and *S. maltophilia* SelTE02 were characterized by the presence of an organic surrounding material
- ▶ Different modifications of the SeNPs extraction protocol acting on this organic material resulted in a strong **variation of the NPs themselves**
- ▶ Our results suggested how **the organic material in which SeNPs are embedded is not covalently bound to the NPs**, considering that the tested treatments were able to rip it off from SeNPs
- ▶ The **organic material surrounding biogenic SeNPs seems to play a key role in the stabilization of the produced NPs**, avoiding the formation of big Se-clusters or Se-aggregates

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Dr. Emanuele Zonaro
Alessandra Bulgarini



**Doc. Raymond Joseph Turner and Microbial
Biochemistry Laboratory (University of
Calgary)**

Dr. Alessandro Presentato



THANK YOU FOR YOUR ATTENTION

