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SUPPLEMENTARY MATERIAL

Critical evaluation of sample preparation for SP-ICP-MS determination of selenium nanoparticles in microorganisms - focus on yeast

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SI-1. Preparation of model samples

Three batches of commercially available baker's yeast *Saccharomyces cerevisiae* were enriched with selenium according to procedures that differed in terms of a growth medium, Se concentration added and time; the resulting preparations have been referred to as Y-A, Y-B and Y-C. The yeast growth and selenium enrichment conditions were aimed at the formation of SeNPs; they are summarized in Table SI-1. Sample Y-D was a commercial Se-rich yeast where the presence of SeNPs was previously detected [unpublished]. Two growth media were used: (1) YPD broth (containing 20 g L^{-1} of bacteriological peptone, 20 g L^{-1} of glucose and 10 g L^{-1} of yeast extract) used for samples Y-A and Y-C and (2) glucose 20 g L^{-1} for sample Y-B. The YPD broth is a common medium for yeast cultivation, either as a commercial product or as an in-house prepared mixture [1, 2]. The substrates for the growth medium were dissolved in water. Fresh baker's yeast was weighed, added to the growth medium and placed in a water bath for 24 h at 32 °C with continuous shaking at 60 rpm. Then, the yeast sample was centrifuged for 10 min at 2000 rpm in 50-mL tubes, the supernatant was discarded, and the pellet was washed with water; the process was repeated twice. The final pellet was weighed to assess the increase in biomass, re-suspended in a 2 mM SeO_3^{2-} solution in water and shaken at room temperature at 60 rpm for 24 to 72 h, after which the color of the yeast suspension turned red. The yeast was centrifuged for 10 min at 2000 rpm and washed with water 3 times to remove the soluble Se. The obtained pellets of selenized yeast and the sample Y-D were freeze-dried at –60 °C for 24 h and stored in sealed polypropylene tubes at 4 °C until the analysis.

Table SI-1 Growth conditions for in-lab cultivated yeast

Yeast sample	Growth medium, concentration [g L^{-1}]	Cultivation time [h]	Mass increase [%]	Mass of SeO_3^{2-} per g of yeast in solution [mg]	Time of incubation with SeO_3^{2-} [h]
Y-A	YPD, 50	24	290	8.1	24
Y-B	glucose, 20	24	42	3.3	24
Y-C	YPD, 50	24	195	8.7	72

Two portions of baker's yeast, purchased from a local grocery store were cultivated in YPD medium and one portion in glucose. After 24 h of incubation, an increase of the biomass was observed in all the cases (Table SI-1). The mass increase of samples cultivated in the nutrients-rich YPD broth (Y-A and Y-C), was found to be significantly higher (195–290 %) in comparison with the yeast cultivated with glucose only (42 %). The yeast after cultivation was washed 3 times with water and centrifuged to eliminate the remaining growth medium from the yeast suspension. The organic molecules present in the medium, such as glucose and proteins, can reduce the Se(IV) to elemental Se and thus create SeNPs, which may affect the results. The washed yeasts were reconstituted with a 2 mM Se(IV) solution and incubated for 24 or 72 h (Table SI-1). Few hours after the addition of Se(IV) the color of the suspensions started to turn red. In addition, yeast Y-C presented a slightly more reddish hue than Y-A, due to the longer period of incubation with Se(IV) and the generation of a higher number of larger NPs. The sample Y-B presented vibrant red color, probably due to a different mechanism of SeNPs production resulting from the previous growth in glucose medium instead of the nutrients-rich YPD broth, although the total Se content was similar in Y-B and Y-C. The commercial selenized yeast (sample Y-D) presented a barely noticeable red hue, which might be due to the presence of low amounts of

SeNPs. The effect of millimolar concentrations of Se(IV) on microorganisms is species-dependent but in many cases a significant growth inhibition was observed for fungi: 89% for *A. pullans*, 97% for *M. humilis* or 82% for *P. glomerata* [3].

SI-2 Total selenium determination and speciation analysis of yeast samples

SI-2.1. Acidic digestion for the total Se determination

The total content of selenium in yeast and selenium nanoparticles suspension was determined by ICP-MS after acidic digestion in a DigiPREP digestion system (SCP Science, Quebec, Canada) in 15 mL DigiTUBES. A mixture of 250 µL of HNO_3 and 50 µL of H_2O_2 was added to 10 mg of dried yeast, and a mixture of 1 mL of HNO_3 and 250 µL of H_2O_2 was added to 250 µL of SeNPs suspension. The digestion temperature was set to 65 °C, and time to 4 h. After digestion the samples were diluted 1000-fold with 2% HNO_3. All samples were digested in duplicates. Procedural blanks were run in parallel.

SI-2.2. Determination of total selenomethionine (SeMet)

0.2 g of sample was incubated with 5 mL of a protease XIV solution (20 mg protease in 30 mM TRIS buffer, pH 7.5). Three consecutive incubations (17 h at temperature 37°C) with fresh portions of the enzyme solution were carried out. After each incubation, the sample was centrifuged (4000×g, 4°C, 10 min), in a centrifuge 5804R (Eppendorf, Hamburg, Germany) and the supernatant was transferred to a separate vial to which 5 µL of β-mercaptoethanol was added. Upon the completion of the whole series, the three supernatants were pooled together and analyzed by anion exchange HPLC - ICP MS in gradient elution mode. Buffer A was A: 20 mM acetic acid – 10 mM triethylamine (pH 4.7) and Buffer B: 200 mM acetic acid – 100 mM triethylamine (pH 4.7). The program was: 0-5 min: 0% B, 5-30 min: 0- to 100% B, 30-35 min at 100% B, 35-36 min to 100%A and 36-40 min at 100% A. The quantification was carried out by the method of standard additions with SeMet at four levels. The samples were analyzed in triplicate. The analytical blanks were included in the measurements. SELM-1 was analyzed in parallel.

SI-2.3. Determination of protein selenocysteine (SeCys) and protein-bound inorganic Se

0.2 g of sample was leached 3 times with a fresh portion 5 mL of water (by sonication during 1 h and centrifugation at 4000×g) in a centrifuge 5804R (Eppendorf, Hamburg, Germany); the solution after each washing was discarded. 2 mL of 0.1 M TRIS buffer (pH 7.5) was added to the residue followed by addition of 30 µL of dithiothreitol (DTT) solution (0.2 M solution in 0.1 M TRIS buffer, pH 7.5) and 50 µL of iodoacetamide (IAM, 0.5 M solution in TRIS buffer). Then, a fresh 150 µL aliquot of the DTT solution was added and the mixture was shaken for 1 h in order to destroy the excess of IAM. Subsequently, 10 mL of TRIS buffer and an aliquot of 1 mL of a protease solution (30 mg protease XIV in 2 mL of 100 mM TRIS buffer, pH 7.5) was added to the sample. After 2 h incubation, a second aliquot of 1 mL protease solution was added and the sample was incubated overnight at 37°C. After centrifugation, the supernatant was removed, filtrated on 2 kDa filter and analysed by HPLC-ICP MS. An Agilent 1200 HPLC system (Agilent, Tokyo, Japan) used as a delivery system for RP was coupled with an Agilent 7700 ICP-MS instrument (Agilent, Tokyo, Japan) fitted with Pt cones and a 1 mm i.d. injector torch.using chromatographic conditions given in Table 1. The quantitation of SeCys and SeMet was carried out using the method of standard (SeCys-CAM and SeMet, respectively) additions at three levels. The samples were analyzed in duplicate. The analytical blanks were measured in parallel.

Table SI-2. Instrumental settings parameters for SP-ICP-MS

Instrumental parameter	
RF Power (W)	1550
Argon flow rate (L min^{-1})	
Plasma	15
Auxiliary	0.9
Sample	1.0
H_2 flow rate (mL min^{-1})	5.0
Sample uptake rate (mL min^{-1})	0.27–0.31
Spray chamber temperature (°C)	2
Dwell time (µs)	100
Readings per replicate	600,000
Total acquisition time (s)	60
Monitored isotope	^{80}Se
Wash-in, wash-out times (s)	30

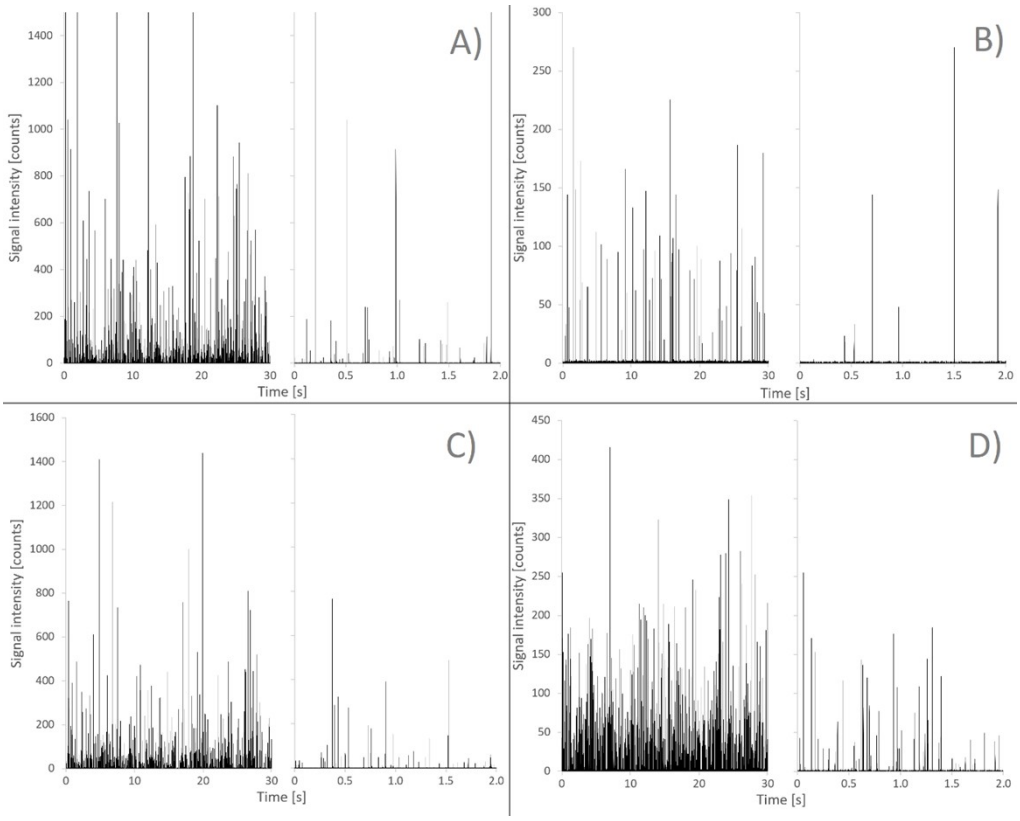

Fig. SI-1. Time scans of ^{80}Se in samples: A) SeNPs after enzymatic procedure (1.61×10^8 particles/L, 1,000-fold dilution), B) SeNPs after mechanical procedure (1.46×10^7 particles/L, 10,000-fold dilution), C) yeast Y-B after enzymatic procedure (1.48×10^8 particles/L, 10,000-fold dilution), D) yeast Y-B after mechanical procedure (1.86×10^8 particles/L, 10,000-fold dilution).

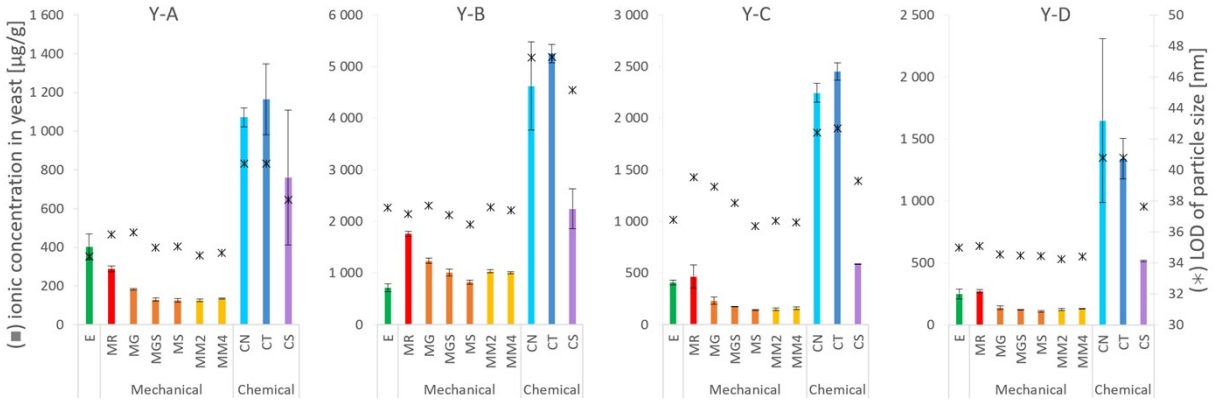

Fig. SI-2. Ionic concentration of Se per g of selenized yeast, and the LOD values, after extraction procedure: E – enzymatic, [4]MR –mechanical [5], MG – mechanical with glass beads, MGS – mechanical with glass beads and sand, MS – mechanical with sand, MM2 – mechanical with 2 metal beads, MM4 – mechanical with 4 metal beads, CN – chemical with 1000 mM NaOH, CT – chemical with 4% TMAH, CS – chemical with 4% SDS. Each column and error bar represents average and SD of 2 replicated extractions.

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