Elucidation of the mechanisms underlying fermentative production of selenium nanoparticles by microorganisms

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Research objective

Selenium is an essential trace element for many organisms, including mammals and bacteria, but it is excessively toxic. In recent years, selenium nanoparticles (SeNPs) have attracted attention due to their lower toxicity compared with other selenium forms as well as their antibacterial, antioxidant, and anticancer activities, which make them promising candidates for applications in medicine, agriculture, and industry [1,2]. Some bacteria reduce toxic selenite to relatively non-toxic SeNPs through fermentation. Notably, bacterial SeNPs are coated with organic materials, including polysaccharides, proteins, and lipids, which confer them properties that are distinct from those of chemically synthesized SeNPs, such as enhanced biocompatibility. However, the mechanisms through which bacteria produce SeNPs with these organic materials incorporated remain largely unknown.

In this study, we aimed to elucidate the mechanisms underlying SeNP formation in two bacterial strains, *Escherichia coli* BW25113 and *Cellulomonas* sp. D3a, that produce SeNPs with distinct characteristics.

Methods and Results

1. Study on the mechanism of formation of extracellular selenium nanovesicles in *E. coli*

We have observed that extracellular SeNPs produced by *E. coli* are enclosed within the cell membrane and exist as selenium nanovesicles (SeNVs). However, SeNVs have not been reported, and their underlying mechanisms remain unknown. Therefore, we cultured *E. coli* deletion mutants of genes involved in cell membrane integrity (Δ*ompC*, Δ*ompA*, Δ*nlpI*, and Δ*tolA*) in the presence of selenite to examine their ability to form SeNPs. The results showed that both wild-type (WT) and mutant strains reduced selenite and produced SeNPs. However, the efficiency in SeNP formation differed between the strains.

Phase-contrast microscopy and transmission electron microscopy (TEM) revealed that, in the Δ*ompC* and Δ*tolA* strains, the extracellular release of SeNPs was strongly impaired, with many SeNPs accumulating in the cytoplasm (Fig. 1). Furthermore, the intracellular SeNPs

formed in these mutants were significantly larger in diameter than those formed in the WT strain.

We also compared the resistance to selenite of the WT, Δ*ompC*, and Δ*tolA* strains, and found that the Δ*ompC* and Δ*tolA* mutant strains exhibited lower resistance to selenite compared with the WT (data not shown).

These results suggest that the extracellular release of SeNPs in *E. coli*, that is, the formation of SeNVs through the cell membrane, requires the integrity of the membrane structure, including OmpC and TolA.

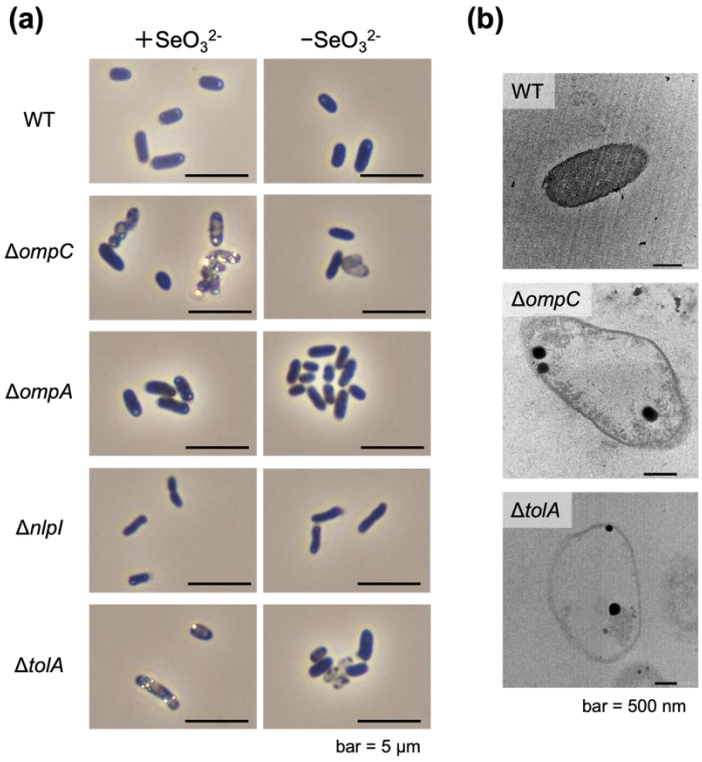

Fig. 1. SeNP formation in *E. coli*. (a) Phase-contrast microscopy images of WT and mutant strains cultured in the presence and absence of selenite. (b) TEM images of ultrathin sections of WT, Δ*ompC*, and Δ*tolA* strains cultured in the presence of selenite.

2. Study of the intracellular aggregation mechanism of SeNPs in *Cellulomonas* sp. D3a

Cellulomonas sp. D3a, which was isolated from a selenium-accumulating soil in India, exhibits a high capacity for selenite reduction. SeNPs produced by this strain accumulate intracellularly, reaching particle sizes of up to 500 nm. When cultured in the presence of selenite, the strain reduces selenite during growth, resulting in the formation of both small SeNPs that aggregate into Se^0 clusters and large spherical SeNPs (sSe^0).

To elucidate the formation process of sSe^0, time-course observations were performed using TEM, and the proportions of different particle types were quantitatively evaluated. Between 3

and 6 h of cultivation, small sSe^0 and Se^0 clusters were observed. From 9 to 24 h, the proportion of Se^0 clusters decreased, whereas the proportion of sSe^0 particles, approximately 400 nm in diameter, increased (Fig. 2).

Notably, confocal microscopy revealed that the strain was capable of division and growth even when the sSe^0 particles occupied approximately half of the cell volume (data not shown).

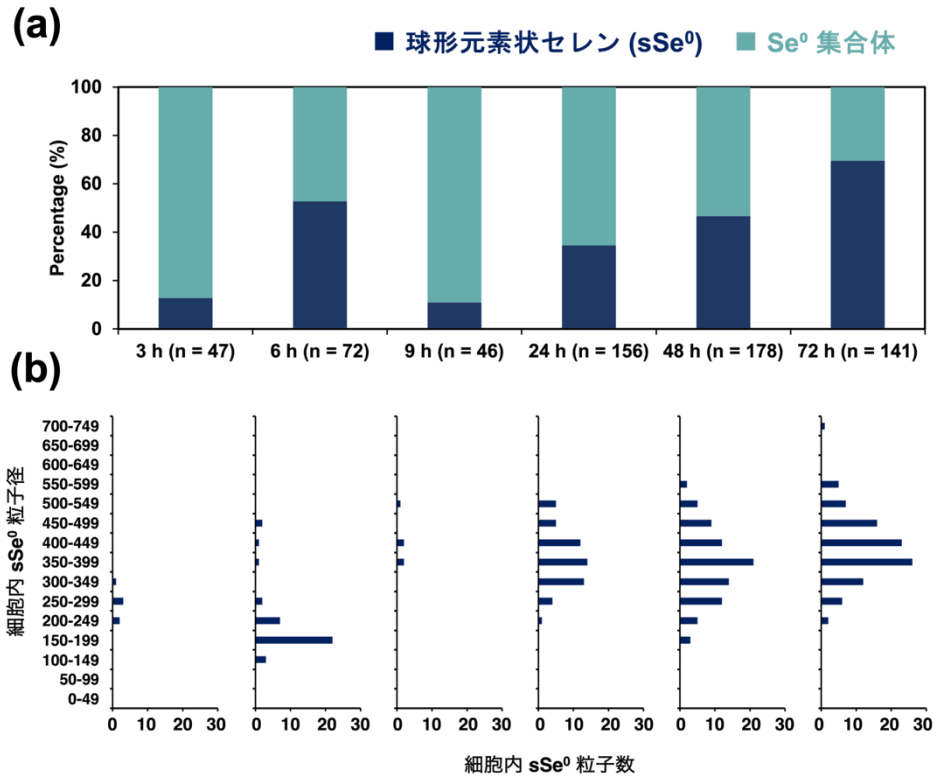

Fig. 2. Intracellular aggregation of SeNPs in *Cellulomonas* sp. D3a. (a) Proportion changes among intracellular sSe^0 and Se^0 clusters over time. (b) Changes in the diameter of intracellular sSe^0 particles over time.

Conclusion

In *E. coli*, the extracellular release of intracellularly formed SeNPs requires the integrity of the membrane structure, including OmpC and TolA, and SeNPs are secreted outside the cell as membrane-enclosed SeNVs. Furthermore, the Δ*ompC* and Δ*tolA* strains exhibited lower resistance to selenite, suggesting that the extracellular release of SeNPs alleviates cellular toxicity. In *Cellulomonas* sp. D3a, SeNPs accumulated intracellularly, and TEM observations revealed that small SeNPs aggregated to form large sSe^0 particles. Furthermore, sSe^0 accumulation did not affect cell division or bacterial growth.

References

1) Sans-Serramitjana, E. *et al*. (2023) Antimicrobial activity of selenium nanoparticles

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2) Cremonini, E. *et al*. (2016) Biogenic selenium nanoparticles: characterization, antimicrobial activity and effects on human dendritic cells and fibroblasts. *Microb. Biotechnol.* ***9*****:** 758–771.