乳酸菌*Lactobacillus casei*の脂質による金属ナノ粒子の合成
Synthesis of metal nanoparticles by glicolipids of *Lactobacillus casei*

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Nanoparticles are small particles with a size of 1-100 nm. They have various unique properties such as quantum size effect, because they differ from bulk crystals. The gold nanoparticles (AuNPs) showed wine-red in color, because of surface plasmon resonance (SPR). Over the past two decades, such nanoparticles have found novel applications in general industry, chemistry, biology, and medicine. To synthesize the AuNPs, the reducing agents give an electron to gold ions , while the dispersing agents inhibit the aggregation of particles to keep the small size. The large amounts of AuNPs are synthesized in the reaction of gold ions with citric acid under conditions of high temperature and pressure in industry. Recently, it is reported that many microorganisms induced to synthesize AuNPs and another metallic nanoparticles. It is attracting a great deal of attentions to synthesize AuNPs at normal temperature under normal pressure and without any toxic byproduct using microorganisms or substance derived from microorganisms. To improve the efficiency of such synthesis, to clarify the molecular mechanisms is required. However, reducing agents and dispersants are unlear. In this study, we approached synthesis of AuNPs by *Lactobacillus casei*. *L. casei* is less harmful for human as used in the manufacture of food such as yogurt, and they have strong resistance for acid.

We synthesized AuNPs using tetrachloride gold (III) acid potassium (auric acid solution) with suspension of *L. casei* and leachate from *L. casei*. After the addition of gold acid solution, the solution turned purple because of surface plasmon resonance of AuNPs. Observations of transmission electron microscope (TEM) showed that black spots of several tens of nanometers were observed. Analyses of ImageJ revealed that the size value of the highest frequency was about 30 nm. Energy dispersion spectroscopy (EDS), attachment of TEM, showed that the nanoparticles were composed of Au. X-ray diffraction (XRD) measurements on dried *L. casei* added gold acid revealed several peaks corresponding to gold crystals with a face-centered cubic structure. These results indicated AuNPs formation by *L. casei*.

Making comparison the suspension of *L. casei* added gold acid (Au(+)) and *L. casei* not added gold acid (Au(-)) between before and after the addition of gold acid, we recognized that the lipid was significantly reduced after the addition gold acid. To identify of the lipid reduced, we extracted lipids from *L. casei* and analyzed the lipids using by ^{1}H-NMR and mass spectroscopic analysis. Comparison of the ^{1}H-NMR spectra of Au(+) and Au(-) suggested that particular lipids may have been associated with AuNPs synthesis. Next, we subjected both lipid extracts to two-dimensional thin-layer chromatography (TLC). Comparing the spot patterns, we found the spot that vanished after adding gold acid, so we scrapped off the spot from the TLC plate and subjected to matrix-assisted laser-desorption ionization–time-of-flight mass spectrometer (MALDI-TOF-MS). This analysis showed that the component of the spot of TLC is diglycosyl diacyl glycerol (DGDG) with two unsaturated fatty acids, and there are DGDG with different length of carbon chains.

To check whether DGDG can synthesize AuNPs *in vitro*, we added gold acid to DGDG extracted from *L.*

casei. After the addition, the color of solution turned red. Observation by TEM and analysis by EDS showed the AuNPs synthesis. Similarly, synthesis of AuNPs could be reproduced by adding to commercially available DGDG. On the other hand, only the sugars and fatty acids that configure the DGDG could not induce the formation of gold nanoparticles at the same concentration of DGDG. These results suggested that glycolipids, such as DGDG, play important roles in reducing Au(III) to Au(0) and in ensuring that the nanoparticles synthesized remain small in size. Moreover, we synthesized palladium nanoparticles by adding tetrachloride palladium(II) acid potassium to DGDG. This suggest that DGDG may have potential to synthesize various metal nanoparticles.

This research is expected to lead to the development of a method of efficient synthesis of gold and another nanoparticles by microorganisms.

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