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Impact of surface-functionalized polystyrene latex nanoparticles on the growth of *Methanosarcina barkeri*

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ABSTRACT

The impact of surface-functionalized polystyrene latex (PSL) nanoparticles (NPs) on *Methanosarcina barkeri* was assessed under anaerobic conditions. *M. barkeri* was cultured in DSM 120 medium in the presence of PSL NPs. As a result, amine-modified PSL NPs (PS-NH_2) inhibited growth, whereas carboxylate-modified PSL NPs had no negative impact on growth. Interestingly, confocal observation revealed that the cells were alive even though the methane was hardly generated and no evidence of NP adhesion on the cell surfaces and NP internalization in the cells were identified. This is because the zeta potential of PS-NH_2 decreased with an increase of the concentrations of yeast extract and Casitone in the DSM120 medium and the polarity changed from positive to negative. In addition, removal of yeast extract and Casitone from the DSM 120 medium induced the precipitation of phosphate compound containing calcium and magnesium. From the above experimental results, when PS-NH_2 are added to DSM 120 medium, the phosphorus required for cell growth forms a phosphate compound on the PS-NH_2 surface due to adsorption of components stabilizing calcium and magnesium in the medium, as a result of which the phosphorus source is deficient and growth is inhibited.

1. Introduction

Nanoparticles (NPs) of one-dimensional particle size within the range of less than 100 nm are of increasing interest due to their unique physicochemical properties, including enhanced catalytic, electrical, optical and magnetic properties, which differ from those in the corresponding bulk material [1–5]. Consequently, changes in physical properties will increase the versatility and effectiveness of product development, leading to more diverse and effective industrial applications [6]. In contrast, significant advances in surface functionalization have enabled control of NPs for beneficial medical applications [7–9]. In addition, nanotechnology applications are already used in various consumer products, and the number of commercial products has increased rapidly [10]. Engineered NPs enable specific physical or chemical interactions with their environment, and these specific characteristics can lead to harmful interactions with biological systems [11,12]. The potential adverse impacts of NPs have not been well understood [13]. In addition, NPs and dissolved ions from NPs can have toxic effects on microorganisms or plants in soil and water [14]. Ultimately, higher species such as humans may be exposed to NPs via bioaccumulation through the food chain [15,16]. Thus, the potential adverse impact of NPs on the environment and human health must be addressed.

Most of the engineered NPs released from consumer products will eventually accumulate in wastewater treatment plants through washing [17,18]. In the plants, wastewater treatment is carried out using various type of microorganisms. Thus, it is extremely important to examine the effect of accumulated NPs on microorganisms. In our previous studies, evaluation of nanotoxicity to prokaryotic bacteria and eukaryotic fungi was studied using polystyrene latex (PSL) NPs as a model surface-functionalized polymeric NP [19–23]. We demonstrated that the positively charged PSL NPs cover the surface of microbial cells by electrostatic attraction, leading to cell death. However, the nanotoxicity to archaea and anaerobic microorganisms had not yet been studied. While intensively studies have been carried out on the toxicity of NPs under aerobic conditions, more research is required to address their potential adverse effects in anaerobic conditions [24]. In anaerobic digester, it is known that the methane production process is the rate limiting step due to the slow growth rate of methanogen [25]. In this study, the impact of polymer NPs on methanogen was assessed under anaerobic conditions. The surface-functionalized PSL NP was used as a model polymeric NP. A pure culture of *M. barkeri* was used as a model anaerobic archaea.

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Table 1
Addition of 0.5 mL solution containing specific medium components to supernatant medium after removal of PS-NH_2 from DSM 120 medium (mg).

Run	A	B	C	D	E	F
K_2HPO_4	0	0.908	0	0.908	0.908	0.908
KH_2PO_4	0	1.392	0	1.392	1.392	1.392
NH_4Cl	0	2.0	2.0	0	2.0	2.0
$MgSO_4{\cdot}7H_2O$	0	2.0	2.0	2.0	0	2.0
$CaCl_2{\cdot}2H_2O$	0	1.0	1.0	1.0	1.0	0

2. Experimental

2.1. Polystyrene latex nanoparticles

Two types of PSL NPs with a fluorophore (nominal diameter: 100 nm) were used. Amine-modified PSL NPs were purchased from Sigma–Aldrich (L9904, St. Louis, MO, USA) and named PS-NH_2. Carboxylate-modified PSL NPs were purchased from micromod Partikeltechnologie GmbH (29-02-102, Rostock, Germany) and named PS-COOH. The PSL NPs were suspended in sterilized pure water in the range 0–40 μg/mL using a vortex for 10 s prior to use. At the neutral pH, PS-NH_2 and PS-COOH were charged positive and negative, respectively.

2.2. Strain and growth conditions

Methanosarcina barkeri JCM10043 was purchased from the Japan Collection of Microorganisms. The growth method of *M. barkeri* is as follows. DSM120 medium (38.6 mL) was added to a 120-mL pressure culture bottle. The components of the DSM120 medium are listed in **Tables 1–3S**. The pH of the medium was 6.8 and 0.5% (v/v) methanol was added as a substrate. The bottle was sealed with a butyl rubber stopper and an aluminum crimp seal and was then degassed using a deoxygenized gas pressure injector (IP-8, Sanshin, Yokohama, Japan) with an oxygen-free N_2/CO_2 gas mixture (80:20) at 120 kPa. After ensuring anaerobic conditions, the bottle was autoclaved at 121 °C for 20 min. After that, $NaHCO_3$ solution (0.4 mL, 8.5% (w/v)) was dispensed into the bottle using a syringe with a sterilized 0.2 μm filter on a clean bench. The next day, after checking that there was no contamination and sedimentation, the precultured *M. barkeri* (1 mL) was inoculated into the bottle using a syringe and was anaerobically grown without shaking at 37 °C.

2.3. Influence of PSL NPs on the growth of M. barkeri

DSM 120 medium (3.8 mL) was taken from the 120-mL pressure culture bottle using a syringe and was dispensed into a 32-mL pressure culture tube. The tube was sealed with a butyl rubber stopper and a plastic threaded cup and was then degassed with an oxygen-free N_2/CO_2 gas mixture (80:20). PSL NP suspension (0.1 mL) was dispensed into the tube using a syringe. Subsequently, the cell suspension (0.1 mL, 2×10^5 cells/mL) was inoculated and incubated at 37 °C without shaking. The final concentration of PSL NPs varied from 0 to 40 μg/mL. Methane production was measured using a thermal conductivity detector for gas chromatography (GC-8APT, Shimadzu, Kyoto, Japan). The optical density of the culture was measured at 660 nm using a digital colorimeter (Mini Photo 10, Sanshin). Before inoculation, precultured *M. barkeri* was collected and washed with a sterilized physiological saline to remove medium components and metabolites. The cells were then re-suspended in physiological saline and the cell concentration was adjusted to 2×10^5 cells/mL.

2.4. Influence of medium components of the growth of M. barkeri

DSM 120 medium (39 mL) and PSL NP suspension (0.1 mL, final concentration: 40 μg/mL) were added to a 120-mL pressure culture bottle and then degassed with an oxygen-free N_2/CO_2 gas mixture (80:20). The bottle was allowed to stand at 37 °C. After 3 d, the supernatant (3.4 mL) was transferred to a 32-mL pressure culture tube. Then, a solution (0.5 mL) containing specific medium components (Table 1) was dispensed and then degassed with an oxygen-free N_2/CO_2 gas mixture (80:20). After that, the cell suspension (0.1 mL, 2×10^5 cells/mL) was dispensed into the tube using a syringe and incubated at 37 °C without shaking. The optical density of the culture was monitored.

2.5. Confocal observation

Confocal laser scanning microscopy (CLSM; FV-1000D, Olympus, Tokyo, Japan) with an oil-immersion objective lens of N.A. = 1.4 (UPLSAPO 100XO) was used to determine the location of the PSL NPs and the cell viability of *M. barkeri* after exposure to PSL NP suspension. Dead cells were stained by membrane-impermeable propidium iodide (PI) (Molecular Probes, Eugene, OR, USA), which only infiltrates cells with disrupted membranes. All cells were stained by membrane-

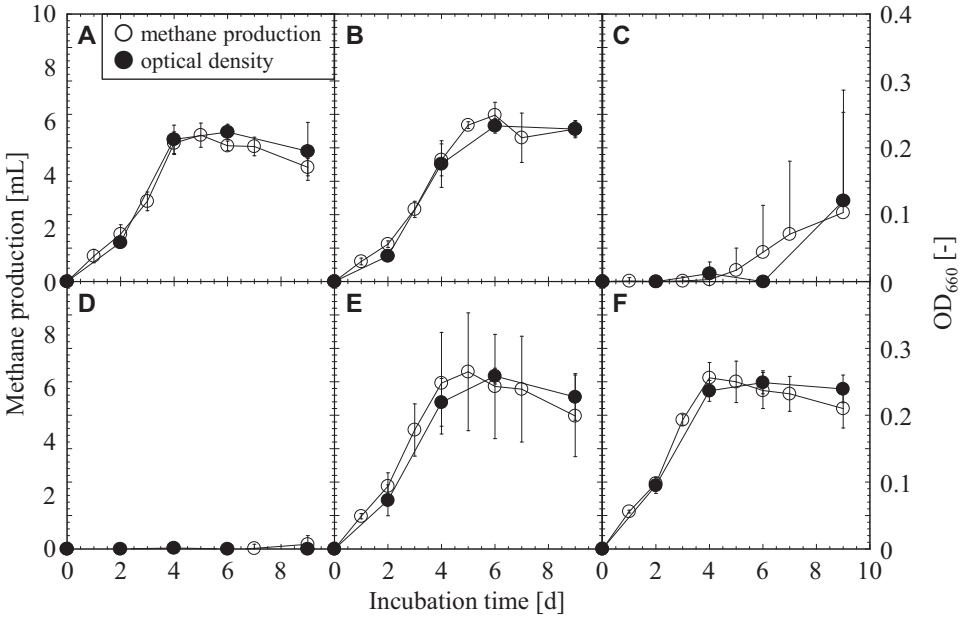

Fig. 1. Growth curves of *M. barkeri* in the presence of PSL NPs: (A) 10 μg/mL PS-NH_2, (B) 15 μg/mL PS-NH_2, (C) 20 μg/mL PS-NH_2, (D) 40 μg/mL PS-NH_2, (E) 40 μg/mL PS-COOH and (F) Control (without PSL NPs). Open symbols represent methane production and closed symbols represent optical density. Mean ± standard error, $N = 3$.