Enrichment of Uncommon Bacteria in Soil by Fractionation Using a Metal Mesh Device

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The use of a metal mesh device (MMD) as a precision bacterial separation filter is described. The MMD uses a structure in which identically shaped pores are arranged in a thin metal membrane. Four types of MMD with different pore sizes were used to fractionate bacteria in two types of soil. Through metagenomic analysis, the distribution of bacteria in the soil samples and in each MMD fraction was examined. In addition, eight types of previously described organic compound-degrading bacteria were used to evaluate the method, and changes in their composition following MMD fractionation were investigated. It was found that MMD fractions were enriched for all eight bacteria when compared with the initial sample. These results suggest that bacterial fractionation using MMD can enrich bacteria occurring at low frequencies in environmental samples.

Keywords Metal mesh device, bacteria, fractionation, enrichment

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Introduction

A metal mesh device (MMD) has a periodic structure in which a pattern of pores with the same shape is arranged in a thin metal membrane. The MMD has optical properties corresponding to this pattern and acts with a band pass filter-like property that transmits only a specific frequency range. In addition, by changing the periodic structure, the frequency of the transmitted electromagnetic band can be altered from the microwave to visible light ranges.[1] As a result, research on quantitative determinations based on the optical properties of MMDs has been pursued. Firstly, Miyamaru *et al*. (2006) suggested that the electromagnetic wave transmission characteristics could be altered based on the amount of material on the surface of the MMD.[2] This was followed in 2013 by Seto *et al*. who reported that quantitation of protein was possible based on the passage of the mid-infrared region of the electromagnetic spectrum through a MMD.[3] More recently, research using MMDs as membrane filters has commenced. Compared with resin-based membrane filters, MMDs have properties that include the following: (1) a uniform pore size to the sub-micron level, (2) the use of low pressure results in less physical damage to the separated material, and (3) since the separated material is arranged on a thin metal film, recovery and analysis are facilitated. By applying these properties, the separation of particulate matter (PM 2.5) has been reported by Seto *et al*. (2014),[4] as has the separation of airborne micro-organisms by Yin *et al*. (2018).[5]

Industrial applications of bacteria have advanced in the fields of medicine, the environment, food, *etc.* However, a comprehensive understanding of bacterial diversity has not been achieved, and it has been estimated that only 1% have been characterized and identified.[6] Therefore, in bacterial research, it is common practice to seek bacteria with desired functions by sampling a large number of environmental samples from soil and other sources. For example, in 2016, Yoshida *et al*. reported that polyethylene terephthalate-degrading bacteria were present in one of 250 environment samples.[7] Many environmental samples must be analyzed to find a minority of samples containing a high concentration of target bacteria that can be isolated. If the concentration of target bacteria in samples is low, then isolation and culture of candidate bacteria requires the processing of an impractically large number of samples. For this reason, it is necessary to identify environmental samples containing a high concentration of the target bacteria.

The objective of this study was to develop a method for enriching bacteria present at low concentrations in soil and other environmental samples. Based on this method, we attempted to achieve improved efficiency over conventional culture methods in which many environmental samples are collected, followed by large-scale isolation and culture. We report results with respect to the fractionation of soil bacteria using four types of MMD with different pore diameters applied to two types of soil. Bacterial morphologies are variable, with different sizes and shapes, and there are also differences in the cell wall according to Gram stain characteristics and the presence of extracellular structures, such as pili and flagella. However, MMDs are produced using electroforming technology; thus filter characteristics such as pore size, shape, and arrangement can be controlled at the sub-μm level. Although the maximum pore

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diameter can be controlled in a general resin-based membrane filter, it is not possible to control the entire filter structure, as can be achieved with a MMD. We hypothesized that it would be possible to separate and enrich specific bacteria from a naturally occurring mixture, such as those occurring in soil, by preparing a series of MMDs with defined pore sizes and performing a multi-stage fractionation.

Experimental

Isolate bacteria from soil

Soil sample A was collected from an ornamental garden on the Ito campus of Kyushu University, and soil sample B was collected from the environmental conservation forests of the same campus. Soil was sieved using a stainless-steel sieve with a 4 mm mesh size to remove pebbles, insects, and plant residue, and then 25 g of soil was weighed into a 50 mL centrifuge tube and suspended in 25 mL phosphate-buffered saline (PBS). The suspension was dispersed using an ultrasonic washing device (US-10KS, SND Co., Ltd., Tokyo, Japan) for 10 min. Soil components were separated from the suspension using a centrifuge (CAX-370, TOMY SEIKO Co., Ltd., Tokyo, Japan) for 30 min at 500*g*. The supernatant was collected and used as the soil bacterial suspension.

Structure of the MMD and bacterial fractionation using MMD

Figure S1 (Supporting Information; SI) shows the dimensions of a single unit cell of four types of MMD. Table S1 (SI) shows the values associated with the MMD design. The four types of MMDs were made from nickel and were manufactured using the electroforming method. The MMDs were washed with acetone, ethanol, and water. After drying, both sides of the MMDs were sterilized by ultraviolet irradiation (wavelength: 172 nm).[8] As shown in Fig. S2 (SI), the MMDs are housed in a polyacetal surround and used as a membrane filter with an opening of 6 mm diameter. Sample suspension was transferred to the MMD using a syringe pump (YSP-201, YMC Co., Ltd., Kyoto, Japan) and a polypropylene disposable syringe (Terumo Corp., Tokyo, Japan). Fractionation was performed so that the MMDs were in pore size order of 7, 3, 2, and 1 μm. Firstly, the soil bacteria suspension was passed through MMD_A shown in Table S1. The eluate from MMD_A was passed through MMD_B and so on, with the eluate from MMD_C finally passing through MMD_D. The flow rate was set to 5 mL min^{-1}, and the amount of liquid was adjusted so that the coverage of the opening by the separated product was about 50 – 70%. Assays to measure ATP were performed on each of the MMD filtered suspensions using luminescence reagents (CA2-50, TOYO B-Net Co., Ltd., Tokyo, Japan) and a luminescence analyzer (CL-24U, Churitsu Electric Corp., Aichi, Japan). ATP was measured in one sample taken from each suspension. The suspensions were passed through the MMDs one at a time.

DNA extraction and quantification

To prepare the soil suspension for extraction, 1 mL was centrifuged for 5 min at 4000*g* and supernatant was removed to leave a volume of 200 μL, which was then extracted as one sample. After fractionation, three MMDs were extracted to obtain one sample.

DNA was extracted from the soil bacterial suspension, and bacteria was recovered from each MMD using a DNA extraction kit (NucleoSpin® Soil, Takara Bio Inc., Shiga, Japan) according to the manufacturer's instructions. Sixteen milligrams of skim milk was added to the ceramic bead filled tubes supplied with the kit to suppress non-specific DNA adsorption, followed by addition of the sample to be extracted and 700 μL buffer SL2, which was supplied with the kit. Using a fluorescence-staining reagent (Qubit dsDNA HS Assay kit, Thermo Fisher Scientific, Inc., MA) and a fluorescence emission reader (Qubit, Thermo Fisher Scientific, Inc.), the amount of extracted DNA was measured in triplicate.

Amplification of the 16s rDNA region by PCR

The sequences of the PCR primers (Fasmac Co., Ltd., Kanagawa, Japan) used are shown in Table S2 (SI). To 11.5 μL of extracted DNA, 0.5 μL of both forward and reverse primers (10 μM) and 12.5 μL of reagent (2 × KAPA HiFi HotStart ReadyMix, NIPPON Genetics Co., Ltd., Tokyo, Japan) were added to a final volume of 25 μL. Using a PCR instrument (MiniAmp Plus Thermal Cycler, Thermo Fisher Scientific, Inc.), PCR was performed under the following conditions: 95°C for 3 min followed by 30 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 30 s, and finally 72°C for 5 min.

The quantity of 16s rDNA present was confirmed by agarose electrophoresis. Agarose (Agarose-S, NIPPON Genetics Co., Ltd.) and TAE (Tris–acetate–EDTA) buffer were used to prepare 2% agarose gels. Electrophoresis was performed using a submarine gel electrophoresis tank (Mupid-2plus, Takara Bio, Inc.) after adding loading buffer (Takara Bio, Inc.) to 3 μL of the post-PCR solution. Molecular weight markers (100bp DNA Ladder One, Nacalai Tesque, Inc., Kyoto, Japan) and a control solution (primer only solution) were also used. Ethidium bromide (MP Bio Japan, K.K., CA), adjusted to 0.5 μg mL^{-1} with TAE, was used for staining and image analysis after electrophoresis. Images of bands in the stained gels were taken using an image capture device (AE-6905H, ATTO, Corp., Tokyo, Japan).

Sequence analysis of 16s rDNA by Next Generation Sequencing

Following PCR amplification, magnetic beads (AMPure XP, Beckman Coulter, Inc., CA) were used to purify the extracted DNA solution, and then a capillary electrophoresis device (MultiNA, SHIMADZU, Corp., Kyoto, Japan) was used to confirm the quality of the 16s rDNA fragments. Indexed NGS (Next Generation Sequencing) adapters/primers required for analysis were added to the purified 16s rDNA fragments by PCR. Twelve sets of reverse PCR primers (N701-12, Illumina, Inc., CA) and eight sets of forward primers (S501-08, Illumina, Inc.) were used. To ensure that each sample had a different primer combination, a reaction mixture consisting of 7.5 μL of the purified DNA solution, 2.5 μL of the forward primer solution, 2.5 μL of the reverse primer solution, and 12.5 μL of 2 × KAPA HiFi HotStart ReadyMix were prepared. Eight cycles of the PCR conditions previously described were run. Following magnetic bead purification of amplicons and confirmation by capillary electrophoresis, sequences were determined by NGS sequencing (Miniseq, Illumina, Inc.).

Metagenomic analysis based on 16s rDNA sequence data

The QIIME2 microbiome bioinformatics platform[9] was used to analyze 16s rDNA sequencing data. Sequences were clustered at the specified identity level of >97% to filter out noisy reads. Sequences were clustered to Operational Taxonomic Units (OTUs) based on sequence similarity. For taxonomic analysis, level seven (species) classification was based on referencing the Greengenes database. In addition, principal component analysis was performed to evaluate relationships between samples. QIIME2 results were observed using QIIME2 View (https://view.qiime2.org/).

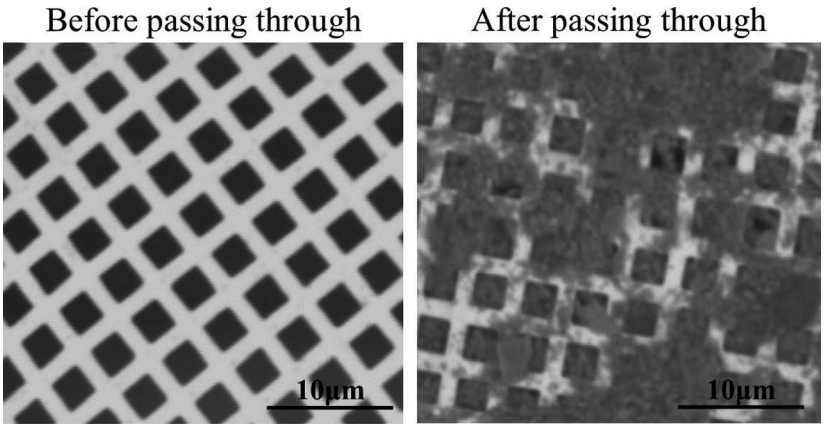

Fig. 1 Comparison of MMD grids before and after suspension was passed through MMD_B.

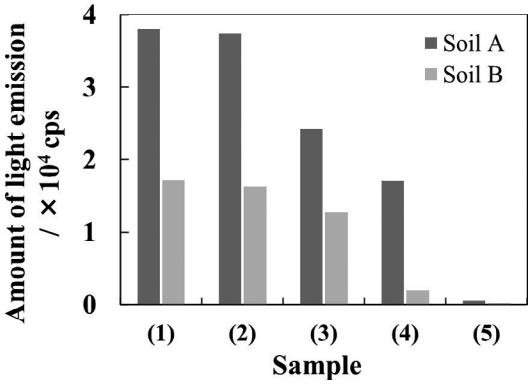

Fig. 2 Measurement of bioluminescence using an ATP assay of each sample. The vertical axis represents the emission intensity. The horizontal axis represents the sample type. (1: Solution of soil bacteria before separation; 2: Solution that passed through MMD_A; 3: Solution that passed through MMD_B; 4: Solution that passed through MMD_C; 5: Solution that passed through MMD_D). Dark gray, soil A; light gray bars, soil B.

Table 1 Amount of DNA extracted from each sample

No.	Amount of DNA/ng μL^{-1}			
	Soil A		Soil B	
	Mean	S. D.	Mean	S. D.
1	4.57	0.46	13.87	0.85
2	2.73	0.24	1.56	0.36
3	2.79	0.09	1.57	0.58
4	2.65	0.27	5.11	0.31
5	1.39	0.36	1.33	0.36

Evaluation of the concentration effect of low-concentration bacteria using MMD

Using previously reported organic molecule-decomposing bacteria as a model, enrichment of bacteria in soil using fractionation by MMD was evaluated. Table S3 (SI) shows the eight types of organic matter-degrading bacteria used as models, the classification of bacteria to genus and phylum-levels, the organic molecules that these bacteria decompose, and references.

Results

Results of soil bacterial fractionation using MMD

Figure 1 shows scanning electron micrographs (TM3030, Hitachi High-Tech Corp., Tokyo, Japan) of MMD grids before and after suspension was passed through. This example shows the results for the suspension of soil A that was passed through MMD_B. These results suggest that, as the solution passed through the MMD, bacteria in suspension were held back on the MMD grid.

Figure 2 shows bioluminescence measurements of the initial bacterial suspensions of soils A and B and suspensions after passing through each of the MMDs. From these results, it was found that bacterial ATP bioluminescence decreased as the fractionation process progressed from MMD_A to MMD_D.

Hereinafter, the sample derived from bacteria in the initial soil bacterial suspension is referred to as sample No. 1, and the samples derived from bacteria isolated at MMDs with pore diameters of 7, 3, 2, and 1 μm are referred to as samples Nos. 2 to 5, respectively. Table 1 shows the mean value and standard deviations (n = 3) for the quantity of DNA extracted from all samples. From these results, it was determined that 1.3 to 13.9 ng μL^{-1} DNA considered to be of bacterial origin was extracted from soils A and B.

PCR amplification of the 16s rDNA region was performed on triplicate DNA samples extracted from all five samples of soils A and B. Following amplification, amplified 16s rDNA fragments were confirmed by electrophoresis. Figure S3 (SI) shows images of the gels after electrophoresis. Two gels (Gel 1 and Gel 2) were used for each of the soils. Unnecessary lanes in the gels were erased and are indicated by white lines. From these results, it was observed that there was a clearly visible band in samples Nos. 1 to 5 close to 400 bp, which represents the molecular weight of the 16s rDNA region.

Next, NGS sequence analysis of 16s rDNA fragments after PCR amplification of the samples was performed. Table 2 shows the mean value and standard deviations for three replicates. Based on these results, the total number of sequences obtained (50000 to 60000), with approximately 1700 to 2300 OTUs, was sufficient to allow comparison of the bacterial flora contents of the samples.

Subsequently, principal coordinate analysis was performed on the distribution of the bacterial flora in soils A and B in samples Nos. 1 to 5, as reflected in metagenomic analysis, to determine relationships between samples. Figure S4 (SI) shows the results

for all samples in triplicate. From these results, it was found that there was a difference in the positions of data for samples Nos. 1 to 5 within the principal coordinates of both soils, reflecting a difference in the composition of the bacterial flora.

Comparison of the bacterial flora assessed at seven levels of taxonomic classification

The distribution of bacterial flora in the samples was compared at seven levels of taxonomic classification and is partially shown in Table S4 (SI). To enable comparison, the most frequent 20 taxa in sample No. 1 were selected and were assumed to represent the dominant bacteria in the respective soils. Taxa belonging to the Archaea are italicized.

Figure 3 shows the changes in the total content rate of these dominant bacteria among the samples. As shown in the figure, in both soils A and B the total content of dominant bacteria was highest in sample No. 1 and lower in samples Nos. 2 to 5.

Enrichment of uncommon bacteria by bacterial fractionation using MMDs

Table S3 (SI) shows eight previously reported organic molecule-degrading bacteria used as a model of bacteria likely to be present at low concentration (content rate 0.1% or less). Changes in the content rate of these eight bacteria at seven levels of taxonomic classification were measured in samples Nos. 1 to 5 from soils A and B. Table 3 shows the proportions of these bacteria in the samples. For each bacterium, the enrichment value "*M*" was calculated as follows;

$$M = C_{max}/C_1$$

where C_{max} and C_1 are the maximum values of content rate in samples Nos. 2 to 5 and the content rate for sample No. 1. When the percentage in sample No. 1 was 0.000%, 0.0004% was used as the provisional percentage as a result of rounding. The calculated *M* values are shown in italics. These results show that percentage values in sample No. 1 were 0 to small, whereas the maximum percentages in samples Nos. 2 to 5 were larger than those in sample No. 1. The results suggest that enrichment occurred following use of the MMDs. Furthermore, it was found that enrichment occurred for the same MMD for

Table 2 Total number of DNA sequences obtained by sequence analysis and number of operational taxonomic units (OTUs) obtained by metagenomic analysis

No.	Soil A				Soil B			
	Total number		OTU		Total number		OTU	
	Ave.	S. D.	Ave.	S. D.	Ave.	S. D.	Ave.	S. D.
1	62366	6581	2349	176	59012	11707	1905	280
2	57484	9970	2179	255	57463	15350	2041	437
3	49848	13225	2086	419	61337	10406	2145	288
4	51894	13440	1838	305	52177	11929	1748	329
5	56306	10262	2073	260	54622	6176	1804	166

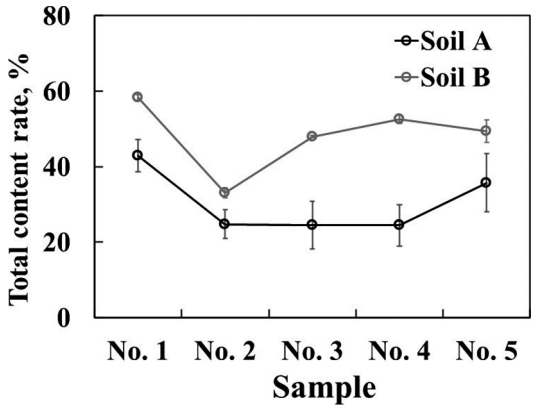

Fig. 3 The mean value and standard deviations ($n = 3$) for the total content rate of bacteria within the top 20 taxa in sample No. 1 of both soil types. The horizontal axis represents the sample type, and the vertical axis represents the total content rate. Black line, soil A; gray line, soil B.

Table 3 Abundance of eight types of organic molecule-degrading bacteria in samples Nos. 1 to 5 (The top row shows the mean value for $n = 3$ followed by the standard deviation in the next row. For each bacterium, the maximum percentage occurrence in samples Nos. 2 to 5 corresponding to the bacterial sample fractionated by MMD is shaded gray.)

Ex.		Soil A						Soil B					
		Content rate/$\times 10^{-3}$%					M	Content rate/$\times 10^{-3}$%					M
		No. 1	No. 2	No. 3	No. 4	No. 5		No. 1	No. 2	No. 3	No. 4	No. 5	
1	AVE.	1	0	3	1	10	**7**	0	2	2	2	7	***17***
	S.D.	2	0	5	3	10		0	3	4	4	7	
2	AVE.	0	100	0	0	10	***251***	6	263	4	2	0	**44**
	S.D.	0	30	0	0	18		10	48	8	4	0	
3	AVE.	0	12	9	8	12	***31***	0	24	7	12	27	***67***
	S.D.	0	4	8	8	2		0	21	12	11	6	
4	AVE.	16	269	43	9	64	**17**	0	67	0	0	0	***167***
	S.D.	15	98	45	16	44		0	28	0	0	0	
5	AVE.	0	5	0	0	0	***11***	0	13	7	2	0	***33***
	S.D.	0	8	0	0	0		0	2	7	3	0	
6	AVE.	1	0	0	0	0	***0***	0	4	0	3	0	***10***
	S.D.	2	0	0	0	0		0	4	0	4	0	
7	AVE.	196	121	434	134	1681	**86**	87	453	884	257	601	**69**
	S.D.	43	208	937	421	3217		15	91	123	407	128	
8	AVE.	0	0	0	0	0	***0***	0	9	3	0	0	***23***
	S.D.	0	0	0	0	0		0	16	5	0	0	

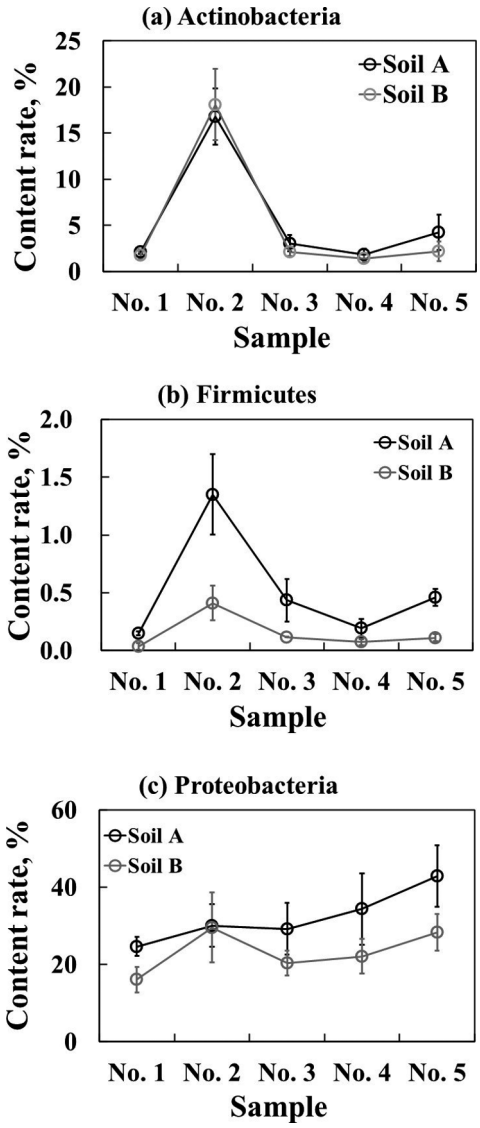

Fig. 4 Changes in the total abundance of bacteria belonging to the three phylum classifications Actinobacteria, Firmicutes, and Proteobacteria in each sample. Panels (a), (b), and (c) show the results for Actinobacteria, Firmicutes, and Proteobacteria, respectively. The horizontal axis represents the type of sample, and the vertical axis represents the content rate obtained from metagenomic analysis. Black curve, soil A; gray curve, soil B.

each bacterium regardless of the soil type, and it was estimated that the maximum enrichment achieved could exceed 100-fold.

Changes in phylum composition resulting from fractionation using MMDs

The eight bacterial genera shown in Table S3 (SI) were divided into three phyla: Actinobacteria, Firmicutes, and Proteobacterium. Using these as examples, changes in the content rate at the phylum level after fractionation using MMD were investigated. Figure 4 shows the changes in the content rate of the three phyla (mean and standard deviation of $n = 3$) in all 10 sample types. The results show that for Actinobacteria and Firmicutes, regardless of soil type, the content rate varied significantly among samples with the maximum abundance observed in sample No. 2. For Proteobacteria, the change in content rate was not as clear, but there was a trend for a gradually increasing percentage in parallel with the increase in sample number. From these results, it was found that there are phyla where the abundance increase occurs in tandem with decreasing pore size and phyla where it does not.

Changes in bacterial flora distribution of bacteria belonging to Actinobacteria

With respect to the Actinobacteria, where a change in abundance occurred with increasing MMD fractionation, changes in the distribution of bacteria belonging to this phylum were investigated. From the results of metagenomic analysis, when the number of bacteria belonging to Actinobacteria was tabulated according to the seven levels of classification, the initial sample (No. 1) contained 30 taxa in soil A and 26 in soil B. In addition, in samples fractionated by MMDs (Nos. 2 to 5) there were 83 taxa detected in soil A and 77 in soil B. Table S5 (SI) lists 53 taxa in soil A and 51 types in soil B that were not detected in sample No. 1. From these results, it was found that approximately 50 taxa of bacteria that were not detected in sample No. 1 were detected in samples Nos. 2 to 5.

Discussions

Bacterial fractionation using MMD

Using a soil bacterial suspension as the initial sample, fractionation with MMDs was performed in order of pore size 7, 3, 2, and 1 μm. By using the data in Fig. 1 (showing before and after passage images of the MMD surface), Fig. 2 and Table 1 (demonstrating ATP and DNA extracted), and Fig. S3 (SI, showing gel electrophoresis images of PCR products), it was determined that the extracted DNA was of bacterial origin. The results clearly indicate that MMD-based bacterial fractionation is possible. Furthermore, Fig. S4 (SI) and Table 2 show that fractionation using MMD can alter the distribution of the bacterial flora recovered.

Enrichment of low-concentration bacteria by bacterial fractionation using MMD

The OTU percentages (showing content rate) provided in this paper were calculated based on the total number shown in Table 2. If the percentage is low and cannot be determined by sampling the total number of bacteria, the percentage is 0.000%. If MMD-based fractionation allows uncommon bacteria to be concentrated and detected within the total bacterial biomass, even if a taxon cannot be detected in a soil suspension, it is possible that it may be detected after MMD enrichment. In Fig. 3, it is shown that the total percentage of each taxon in the initial sample (No. 1), which reflects the most frequently occurring 20 taxa in terms of abundance, decreased in MMD fractionated samples. These results indicate that MMD fractionation increases the proportion of uncommon bacteria, suggesting that MMD is able to enrich bacteria present in low numbers.

Using eight known organic compound-degrading genera shown in Table S3 (SI) as a model for uncommon bacteria, the effectiveness of MMD-based fractionation for enrichment was confirmed. As shown in Table 3, it was found that the same MMD fractions showed higher abundances for each of the bacteria regardless of the soil type. Furthermore, when enrichment was calculated, it was estimated that the concentration was up to 100 times or more. These results further indicate that it is possible to enrich uncommon bacteria in soil using MMD fractionation.

Effect of MMD fractionation at the phylum level

Figure 4 shows changes in abundance after MMD fractionation at the phylum level using three phyla as examples (Actinobacteria, Firmicutes, and Proteobacteria), as these are the most frequently occurring phyla of the eight organic molecule-degrading bacteria used. From these results, it was observed that there was a trend of increased abundance from the enrichment of Actinobacteria and Firmicutes in sample No. 2 regardless of the soil type. However, this effect was not observed in Proteobacteria. One of the reasons for these results is that Proteobacteria is the largest phylum of bacteria and is composed of bacteria with diverse structures. The results in Fig. 4 suggest that it is possible that no clear trend was observed because bacteria with various structures and morphologies were isolated by the use of a MMD. On the other hand, Actinobacteria and Firmicutes have the common feature of being Gram-positive bacteria with a thick cell wall (peptidoglycan layer). Separation of bacteria and cells is not determined solely by size exclusion; for instance, pressure loss during separation can deform bacterial cells and allow them to pass through MMD pores. They may therefore be less likely to deform under pressure due to the thick cell wall, and have a structure that is easily separated using a MMD.

Using Actinobacteria, which showed a trend toward increasing enrichment with decreasing pore size, as an example, changes in the distribution of bacterial flora belonging to this phylum while using MMD fractionation was investigated. As shown in Table S5 (SI), bacteria that were not detected in sample No. 1 but were detected after MMD fractionation included 53 taxa in soil A and 51 in soil B. These data reinforce the suggestion that using MMDs may be able to enrich uncommon soil bacteria.

Conclusions

In this paper, soil bacteria were fractionated from two types of soil using four types of MMD with different pore diameters, and changes in bacterial flora distribution due to the fractionation was investigated. The results demonstrated the efficacy of MMD fractionation. Eight organic molecule-degrading bacteria were used as a model of uncommon bacteria, and the changes to their abundance resulting from fractionation using MMD were evaluated. It was shown that enrichment occurred following fractionation using the same kind of MMD for each bacterium, regardless of the soil type. In addition, it was estimated that the maximum enrichment rate was of the order of 100. We also investigated the enrichment effect of fractionation using MMDs for bacteria belonging to Actinobacteria, which is one of the phyla to which the eight organic compound-degrading bacteria belong. As a result, it was found that approximately 50 species of bacteria that could not be detected in the initial soil suspension sample were detected by fractionation using MMD enrichment. In conclusion, these results suggest that bacterial fractionation using MMDs may be able to enrich bacteria present in environmental samples such as soil.

Conventional environmental bacteriology research requires the collection of a large number of environmental samples to identify those with high concentrations of the target bacteria for isolation and culturing. This process is expensive and time-consuming, and dependent on good fortune, thereby making the discovery of bacteria with desirable properties difficult. We believe that the application of the enrichment method described in this study will increase the likelihood of detecting target bacteria and improve efficiency by reducing the number of environmental samples that must be examined.

Since bacteria and cells are not rigid, pressure at the time of separation can deform them and allow them to pass through pores smaller than their own size. By utilizing this phenomenon, it is possible to perform separation according to differences in cell rigidity (deformability) by controlling the structure of MMD and the fluid conditions. In addition, MMDs can be modified to retain organic molecules such as antibodies on their surfaces.[4,8] By using these modified MMDs, it should be possible to retain certain bacteria on the MMD surface while letting others pass though. In the future, we would like to perform more accurate bacterial fractionation by combining these separation principles together with size exclusion.

Supporting Information

In SI, we show data such as the flora distribution of samples Nos. 1 to 5 of soils A and B. This material is available free of charge at http://www.jsac.or.jp/analsci/.

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