氏　　名	Parmila Kumari
博士の専攻分野の名称	博士（学術）
学位記号番号	博理工甲第 960 号
学位授与年月日	平成 26 年 9 月 19 日
学位授与の条件	学位規則第 4 条第 1 項該当
学位論文題目	Study on the Mutation-causing Effect of Ultra-low (ppb) Concentration Mutagens such as Contained in Drink Water Sample and others （飲料水等を対象とした極低濃度（ppb）変異原の細胞変異原性実証研究）
論文審査委員	委員長　教　授　松岡　浩司 委　員　准教授　石丸　雄大 委　員　准教授　根本　直人 委　員　准教授　幡野　　健 委　員　名誉教授　西垣　功一

論文の内容の要旨

Species identification and classification of a large number of microbes are essential and heavy workloads in culture collections and relevant laboratories. The identification of species usually requires different methods depending on species. So far, several approaches have been developed based on the genomic DNA for this purpose: PFGE (Pulsed-field gel electrophoresis), RAPD (random amplified polymorphic DNA), AFLP (amplified fragment length polymorphism), 18S rDNA sequencing, and others. Unfortunately, none of these methods can escape from the information shortage that leads to a failure in correct identification and classification of species. Therefore, the development of a method which is simple and applicable to any organisms will lessen the burdens, increase the reliability of databases and thus enhance the science on microbes.

In this context, a simple and universal method to identify and classify species has been developed: the Genome Profiling (GP) method, a genotype based technology, which exploits random PCR and temperature gradient gel electrophoresis. Effectiveness of this technology for species identification, classification, and mutation detection has been confirmed.

In my study, after the initial advanced training of GP, where I could demonstrate jointly that only a single run of the GP experiment (i.e., in a rapid and low cost manner) could cluster three different mouse families discriminatively and also cluster siblings of the same parent without *a priori* knowledge.

I proceeded to the next training to screen the effect of some mutagenic chemicals (55 chemicals) using bacterial and 3T3 mammalian cells as test organisms. Our study showed that this approach was not only limited to bacterial cells but could successfully screen the effect of chemicals with the 3T3 mammalian cell also. Results of our study were compared with the Ames test data (bacterial mutagenicity) and other mammalian cell-based mutational studies (Mammalian mutagenicity) and almost complete agreement was observed between mammalian cell-based GPMA and other studies such as Ames test. We found that the GPMA assay of both bacterial and mammalian types can detect the mutagenicity at

the 10 ppb concentration of chemical compounds.

Humans are frequently exposed to thousands of potentially harmful dietary, environmental, and pharmaceutical chemicals, many of which can cause mutagenicity that can lead to cancer in some cases. Once these hazardous substances are widely distributed in the environment, they are difficult to recover and remove and thus have the potential to keep doing harm to human beings and the ecosystem. Potentially hazardous chemicals are therefore used under careful control, and all novel chemicals need to be tested for toxicity, mutagenicity, and carcinogenicity before their general use. Mutagenicity testing is especially important as most mutagens also have carcinogenic properties. Although the causes of carcinogenicity are multiple and complex, it is nevertheless clear that physical and chemical mutagens are significant factors in generating cancers. Physical sources, such as UV rays in sunshine, X-rays used for medical purposes, and radioactivity from environmental contaminants, have the potential to induce cancers in the skin, blood and other organs. A greater risk to human health is from exposure to chemical substances in the environment such as tap water (most abundantly taken food worldwide). There is considerable evidence that a large proportion of human cancer may be caused by exposure to toxic chemicals in the environment, very few of which have been tested for carcinogenicity or mutagenicity. In general, daily exposures to very low doses of mutagen do not induce an immediate response, but exposure over a prolonged period may have an impact and result in cancers and other diseases. Usually, by the Mass spectrometry (MS) analysis the existence of these mutagenic reagents could be detected, although the mutagenicity of them could not be assayed since it was below the detection limit (ppm) of conventional mutation assays such as the Ames test.

Owing to the high sensitivity and proven effectiveness of this technology, we could devise to apply it to determine the mutagenic effect of Japanese drinking water. The pace of developing new chemical reagents is continued to accelerate. These chemicals are under the growing concern worldwide due to their potential to cause cancer, birth deformities, and other serious health issues. As one of the most serious problems in the world, there is an issue of contamination of water from industrial, agricultural, and urban discharges. It has evoked widespread concern over declining water quality, especially when the water body is used as a source of drinking water. Besides, mutagenicity of drinking water is due not only to industrial, agricultural and urban pollution but also to disinfection treatments.

We applied Genome Profiling-based Mutation Assay (GPMA) to detect the mutagenicity of drinking water in Japan without the need to resort to methods involving lyophilization or evaporation to concentrate solutes; these methodological steps are not only time-consuming but also apt to alter or degrade the quality of the samples. In this study none of the mineral water samples (10 samples) were found to exhibit any mutagenic activity and displayed levels equivalent to the control level (*ΔPaSS* of 0.022: the level of spontaneous mutation in *Escherichia coli* cultured for 15 generations in the absence of mutagens); however, all of the tap water samples (142 samples) were significantly mutagenic (although they showed varied level of mutagenicity). In GC-MS analysis, tap water samples were found to contain mutagenic byproducts (THMs; trihalomethanes) at ppb level generated in the water chlorination process. In detail GC-MS analysis, the existence of a trace amount of other possibly mutagenic agents such as benzene, toluene, m, p-xylene, and o-xylene was confirmed but concentration of these mutagenic agents was very low to that of the THMs. Our study showed that THMs were the major mutagenic chemicals present in Japanese tap water and, actually, they contribute to the mutagenicity measured at the intensity of more than a half.

Therefore, the data obtained in this study suggest that GPMA assay can be used as a screening tool for the detection of a low-level (i.e., ppb) contamination of mutagenic chemicals. If the detection of mutagenicity due to the low-level contamination is firmly established using the GPMA analysis, studies on the effect of such low level mutagens exposure

will be specially benefited, thus promoting the cancer research. Hence, the novel approach presented here, GPMA, should be a useful mutation assay that both complements and reinforces the other standard mutation detection assays.

論文の審査結果の要旨

The applicant's dissertation was titled as: *Research on the Mutation-causing Effect of Ultra-low (ppb) Concentration Mutagen using Drinking Water Samples and Others.*（飲料水等を対象とした極低濃度(ppb)変異原の細胞変異原性実証研究）

Species identification and classification of a large number of microbes are essential and heavy workloads in culture collections and relevant laboratories. The identification of species usually requires different methods depending on species. So far, several approaches have been developed based on the genomic DNA for this purpose: PFGE (Pulsed-field gel electrophoresis), RAPD (random amplified polymorphic DNA), AFLP (amplified fragment length polymorphism), 18S rDNA sequencing, and others. Unfortunately, none of these methods can escape from the information shortage that leads to a failure in correct identification and classification of species. Therefore, the development of a method which is simple and applicable to any organisms will lessen the burdens, increase the reliability of databases and thus enhance the science on microbes.

In this context, a simple and universal method to identify and classify species has been developed: the Genome Profiling (GP) method, a genotype based technology, which exploits random PCR and temperature gradient gel electrophoresis. Effectiveness of this technology for species identification, classification, and mutation detection has been confirmed.

At the first stage, the applicant has performed the experiment to screen the effect of some mutagenic chemicals (55 chemicals) using bacterial and 3T3 mammalian cells as test organisms. Her experimental study showed that the GP-based mutation assay (GPMA) approach was effective in both types of experimental organisms (not only bacterial cells but also the 3T3 mammalian cell).That is, the results obtained by those methods were congruent to those examined by the Ames test. The applicant with the help of collaborators found that the GPMA assay of both bacterial and mammalian types can detect the mutagenicity at the 10 ppb concentration of chemical compounds.

Humans are frequently exposed to thousands of potentially harmful dietary, environmental, and pharmaceutical chemicals, many of which can cause mutagenicity that can lead to cancer in some cases. Once these hazardous substances are widely distributed in the environment, they are difficult to recover and remove and thus have the potential to keep doing harm to human beings and the ecosystem. Potentially hazardous chemicals are therefore used under careful control, and all novel chemicals need to be tested for toxicity, mutagenicity, and carcinogenicity before their general use. Mutagenicity testing is especially important as most mutagens also have carcinogenic properties. Although the causes of carcinogenicity are multiple and complex, it is nevertheless clear that physical and chemical mutagens are significant factors in generating cancers. Physical sources, such as UV rays in sunshine, X-rays used for medical purposes, and radioactivity from environmental contaminants, have the potential to induce cancers in the skin, blood and other organs. A greater risk to human health is from exposure to chemical substances in the environment such as tap water (most abundantly taken food worldwide). There is considerable evidence that a large proportion of human cancer may be caused by exposure to toxic chemicals in the environment, very few of which have been tested for carcinogenicity or mutagenicity. In general, daily exposures to very low doses of mutagen do not induce an immediate response, but exposure over a prolonged period may have an impact and result in cancers and other diseases. Usually, by the Mass spectrometry (MS) analysis the existence of these mutagenic reagents could be detected, although the mutagenicity of them could not be assayed since it was below the detection limit (ppm) of conventional mutation assays

such as the Ames test.

Owing to the high sensitivity and proven effectiveness of this technology, we could devise to apply it to determine the mutagenic effect of Japanese drinking water. The pace of developing new chemical reagents is continued to accelerate. These chemicals are under the growing concern worldwide due to their potential to cause cancer, birth deformities, and other serious health issues. As one of the most serious problems in the world, there is an issue of contamination of water from industrial, agricultural, and urban discharges. It has evoked widespread concern over declining water quality, especially when the water body is used as a source of drinking water. Besides, mutagenicity of drinking water is due not only to industrial, agricultural and urban pollution but also to disinfection treatments.

We applied Genome Profiling-based Mutation Assay (GPMA) to detect the mutagenicity of drinking water in Japan without the need to resort to methods involving lyophilization or evaporation to concentrate solutes; these methodological steps are not only time-consuming but also apt to alter or degrade the quality of the samples. In this study none of the mineral water samples (10 samples) were found to exhibit any mutagenic activity and displayed levels equivalent to the control level (*ΔPaSS* of 0.022: the level of spontaneous mutation in *Escherichia coli* cultured for 15 generations in the absence of mutagens); however, all of the tap water samples (142 samples) were significantly mutagenic (although they showed varied level of mutagenicity). In GC-MS analysis, tap water samples were found to contain mutagenic byproducts (THMs; trihalomethanes) at ppb level generated in the water chlorination process. In detail GC-MS analysis, the existence of a trace amount of other possibly mutagenic agents such as benzene, toluene, m, p-xylene, and o-xylene was confirmed but concentration of these mutagenic agents was very low compared to that of the THMs. Applicant's study showed that THMs were the major mutagenic chemicals present in Japanese tap water and, actually, they contributed to the mutagenicity measured at the intensity of more than a half. Therefore, the data obtained in this study suggest that GPMA assay can be used as a screening tool for the detection of a low-level (i.e., ppb) contamination of mutagenic chemicals. If the detection of mutagenicity due to the low-level contamination is firmly established using the GPMA analysis, studies on the effect of such low level mutagens exposure will be specially benefited, thus promoting the cancer research. Hence, the novel approach presented here, GPMA, should be a useful mutation assay that both complements and reinforces the other standard mutation detection assays.

So, the applicant has established the realistic way to measure the mutagenicity of low concentration ingredients such as those of drink waters.