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Genotoxic Effects of Microwave Radiation from Mobile Phones on Bacterial DNA

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ABSTRACT

The potential effects of microwaves on organisms are an area of increasing research interest. Numerous, though conflicting, published studies have addressed this issue. Here, we examined the potential genotoxic effects of frequencies between 800 MHz and 2 GHz microwave radiation associated with mobile phones that use code-division multiple accesses (CDMA) technology. To assess mutagenicity, we used the *umu* test, a modification of the standard Ames test for measuring mutagenicity; the *umu* test provides enhanced sensitivity compared with the Ames standard test. The chosen assay (*umulac* AT-F) utilizes a recombinant *Salmonella* bacterial strain. This bacterium has a recombinant *umuC'-'lacZ* fusion gene; DNA damage enhances the expression of the *'lacZ* enzyme, that subsequently converts a transparent substrate (X-gal) into a blue-pigmented product (indole) that can be detected through colorimetric analysis. Using this assay, we measured DNA damage in bacteria exposed to microwaves emitted from mobile phones for 3 h. Exposure for 3 h was found to be equivalent to the mutagenicity of 1-h exposure to 18 ng/mL and 10 ng/mL furylfuramide (AF-2, a food additive that is potentially carcinogenic), for the receiver side of the mobile phone and the transmitter side of the mobile phone, respectively. Exposure to mobile phones for long periods may have harmful effects on DNA. The convenient detection system used in this study provides a broadly useful approach for investigating both the potentially harmful effects of microwaves as well as microwave-protective products.

1. INTRODUCTION

Microwaves are electromagnetic waves in the frequency range of 300 MHz–300 GHz (1 mm–1 m wavelength). Microwave radiation from space is present naturally in the Earth's atmosphere. In the current environment, additional sources of microwave emissions include everyday items such as microwave ovens and mobile phones. Of these modern technologies that utilize microwaves, the mobile phone may be one of the most popular and universally used devices. Numerous studies have investigated the potential effects of microwave radiation that are emitted from mobile phones on human health; however, this research has produced conflicting results. Some epidemiological studies have indicated harmful effects of mobile phone use on humans, suggesting increased risk of brain tumors [1–3], glioma and acoustic neuroma [4], and malignant melanoma [5]. Microwaves from mobile phone base stations have also been found harmful for general health and salivary function [6]. Some studies have indicated that mobile phones have no significant effects on humans under a certain frequency and duration of product use [7–9]. Beyond studies of mobile phone use, there is a large body of research that indicates the harmful effects of microwave exposure in humans and other organisms. In humans, microwave exposure causes a risk of brain tumors [10], activation of stress pathways in human endothelial cells [11], chromosomal instability in peripheral blood lymphocytes [12], induction of micronuclei in human lymphocytes [13], modification of the hypothalamic–pituitary–thyroid axis [14], inhibition of brain glucose metabolism [15], promotion of cancer growth by overproducing reactive oxygen species (ROS) [16], induction of double-strand DNA breaks in peripheral blood leukocytes and skin fibroblasts [17,18], increased oxidative stress in lens epithelial cells [19], and induction of apoptosis in human fibroblast cells [20].

A substantial number of studies of mobile phone use have documented no significant relationship between mobile phone use and adverse health outcomes. These include studies focused on cognitive function [21]; peripheral blood leukocytes [22]; proto-oncogene or heat-shock protein gene expression in cell lines [23]; micronucleus formation and cell proliferation in peripheral blood lymphocytes [24]; physiological effects including head pain, discomfort, or altered physiological variables [25, 26]; genotoxic effects in leukocytes [27]; cerebral blood flow [28]; micronucleus frequency in buccal mucosa cells [29]; chromosomal aneuploidy [30]; and expression of stress marker proteins such as p53 and hsp70 [31].

Rapidly evolving technologies pose an additional challenge for our understanding of the potential effects of mobile phone use on humans. Models of mobile phones and internet-enabled Smartphone's are frequently updated. Ideally, each time a new model is released, the safety criteria for the technology should be re-examined. However, studies involving eukaryotic cultured cells or organisms are time-consuming and involve considerable costs.

On the other hand, there is scarce research which irradiated microwaves to bacteria. Microwave radiation causes cellular lethality for *Salmonella typhimurium* and *Escherichia coli* [32]. Encouraged by this research, we expected that bacteria could be used for microwave impact assessment. Therefore, in the current study, we intended to utilize the *umu* test as a convenient and efficient method to detect potential carcinogenic effects of microwave radiation from mobile phones and to evaluate the biological effects of this radiation on bacterial genetic material. We documented DNA damage associated with exposure to mobile phones and demonstrated that our simple assay is suitable for the detection of the effects of microwave irradiation. The simple detection system utilized here could also be used to assess the impact of equipment that is designed to protect people from the harmful effects of microwaves emitted from instruments including mobile phones.

2. MATERIALS AND METHODS

2.1. The *umu* test.

The *umu* test is a convenient method to identify the mutagenicity and carcinogenicity of substances. The *umu* test, a variation of the Ames test, utilizes a strain of *Salmonella typhimurium* that contains a recombinant marker gene [33–37]. The *umu* test utilizes the capacity of bacterial cultures such as *Salmonella typhimurium* to restore DNA damage through a process known as the SOS response; the principle of this detection system is described in Figure 1. The test uses a strain of *S. typhimurium* containing a genetically constructed plasmid, pSK1002 (Fig. 2). When the damage to the bacterial DNA is caused by ultraviolet (UV) radiation, chemical substances, or other mutagens, the SOS reaction is initiated, resulting in the expression of the recombinant *umuD*$^{+}$*-umuC'-'lacZ* gene and production of β-galactosidase, the gene product of *'lacZ* (Fig. 2). β-galactosidase (*'lacZ*) converts the colorless X-gal sugar component (galactopyranose) into the blue-colored indole, a product that can be detected using colorimetry (Fig. 3). Therefore, in the *umu* test, the intensity of blue color increases with increasing genotoxicity of the experimental mutagen.

We performed the *umu* test using bacteria and reagents present in a commercial mutagenicity test kit "*umulac* AT (microplate method)" (Iwaki Co. Ltd., Tokyo, Japan). Use of this kit, that contains bacterial cells (NM2009) carrying a genetically modified plasmid DNA, was approved by the bio-risk management committee and gene recombination experiments committee of Osaka Prefecture University, at BSL1 and P1 levels.

2.2. Cell preparation

The frozen test strain of bacteria, *umu*lac AT-F, was thawed and 50-μL aliquots were stored either in liquid nitrogen or in a −80°C freezer until use.

2.3. Positive control

To generate a standard curve using a positive control, one aliquot of *umulac* AT-F was mixed with 1 mL of culture medium from the kit and incubated at 37°C for 1 h. Furylfuramide (AF-2) from the kit was used as a mutagen-positive control. Cultured bacteria (50 µL/well) were dispensed into a 96-well microplate (3860-096; Iwaki Co. Ltd., Tokyo, Japan) and then covered with the plate lid. Serial dilutions of AF-2 in DMSO were prepared and then added (10 µL/well) to each well. The final concentrations of AF-2 were 0.033, 0.1, and 0.3 µg/mL. As a negative control, only DMSO was added to the bacterial cultures. The plate was incubated at 37°C for 1 h. After incubation, the chromogenic substrate X-gal was added (100 µL/well), mixed, and the plate was again incubated at 37°C for 1 h. A stop solution was added (100 µL/well), and the plate was read at a wavelength of 630 nm using a Bio-Rad Benchmark Plate Reader.

2.4. Effects of microwave radiation from mobile phones.

A 50 µL aliquot of *umulac* AT-F was mixed with 0.95 mL of culture medium from the kit and incubated at 37°C for 1 h. A total of 50 µL of the bacterial culture was dispensed in 0.2-mL plastic tubes. Two Smartphone's (iPhone 5c) were set 8 m apart and left on "talk" mode. Two tubes containing the bacterial cultures were wrapped by a 5-mm-thick plastic sheet and attached by tape to the upper side of the transmitting Smartphone's. Two additional tubes containing the bacterial culture were similarly attached by tape to the upper side of the receiving Smartphone's. As a sham exposure control, two more tubes containing the bacterial culture were placed in a 25°C water bath (ThermoMinder SM; Taitec Co. Ltd, Saitama, Japan), that was placed in the middle of the two Smartphone's, thus 4 m apart of both Smartphone's. The intensity of microwaves from the Smartphone's was measured using a Trifield meter (Satoh Shoji, Tokyo, Japan)

To avoid the effects of heat directly radiated from the Smartphone's, the temperature of the surface of the tubes near the phones was constantly monitored using a laser light radiation thermometer BGA B73010 (Biomedical Science, Tokyo, Japan). If the temperatures increased above 25°C, the tubes were cooled by a fan until the temperatures decreased to 25°C. Exposure to Smartphone's radiation was continued for 3 h per experiment. Following the experimental and sham exposures, the six tubes were incubated for 1 h at 25°C. After incubation, the turbidity (OD_{600}) was measured for one of each experimental pair tubes to calculate the concentration of bacteria, as we guessed that some increase in the number of bacteria would occur within the tubes exposed to Smartphone's as a result of the elevated temperatures. A total of 50 μL of the Smartphone's substrate X-gal was added to the other tube of each experimental pair tubes and incubated at 37°C for 1 h. Following incubation, the stop solution was added (50 μL/tube), mixed, transferred into a well of 96 well plate and the plate was scanned at 630 nm using a BIO-RAD Benchmark Plate Reader. The colorimetric values (OD_{630}) of the bacterial cultures exposed to Smartphone's were normalized using the following ratio: (OD_{600} value of exposed control) / (OD_{600} value of sham-exposed control). This was done to compensate for differences in bacterial density among the treatments.

Three independent experiments were performed at separate times, and the average and S.D. of normalized OD_{630} was calculated for each treatment sample. Analysis of significant differences between treatments was performed using an unpaired and non-parametric Student's *t*-test. To compare the mutagenicity of mobile phones with that of AF-2, we applied the difference of normalized OD_{630} between the exposed bacteria and the sham-exposed control on the AF-2 standard curve.

3. RESULTS

3.1. Physical experimental conditions

The intensity levels of microwaves from the mobile phones are described in Table 1. The microwave intensity measured at the midpoint of the two phones (control position) was almost zero, whereas the intensity measured 1 cm from either the transmitting or the receiving phone fluctuated irregularly between 0–1 mW/cm^2.

The average temperatures of the surfaces of the tubes containing bacteria that were placed in front of the Smartphone's and in the interval region (control) have been presented in Table 2. During the experiments, experienced temperatures did not deviate more than 0.6°C from these average temperatures.

3.2. Standard curve of mutagenicity using AF-2

The intensities of normalized OD_{630} increased with increasing concentrations of AF-2 of up to 0.3 μg/mL (Fig. 4). We used the resulting standard curve to estimate the mutagenic potential of Smartphone's.

3.3. Mutagenicity associated with 3-h exposure to Smartphone's

The largest DNA damage effects were observed at the receiver side Smartphone's, with less DNA damage observed on the transmitter side; however, there were no significant differences in DNA damage between both samples placed next to the Smartphone's and the control (sham-exposed) samples. The OD_{630} values (mean ± S.D.) were 0.245 ± 0.062 for controls, 0.290 ± 0.033 for the receiver side Smartphone's, and 0.269 ± 0.067 for the transmitter side Smartphone's (Fig. 5). The effects of 3-h exposures were estimated to be equivalent to that of the treatment for 1 h with 18 μg/mL and 10 μg/mL AF-2 for the receiver and the transmitter

side of the Smartphone's, respectively.

4. DISCUSSION

The documented effects of microwaves on organisms include "Thermal effects," the well-known phenomenon used in microwave ovens, caused by rapidly oscillating water molecules that raise the temperature of organisms. Microwave exposure can also lead to "non-thermal effects" by causing the production of excess ROS and free radicals [12, 36].

The temperature of the main body of a Smartphone's rises while it is charged and on talk mode, causing the temperature of the surrounding air to increase. To avoid increasing temperatures by heat directly irradiated from the Smartphone's, the experiments were performed in a temperature-controlled room set at 25°C. Furthermore, temperature control was maintained by fans and constant monitoring of the surface temperature of the tubes containing the bacterial culture. The volumes of culture medium containing bacteria utilized in the experiments were very small (100 µL). As microwaves are often used for microwave ovens, there could also be thermal effects that could interfere with the study. In fact, a slight temperature change was measured for the receiving Smartphone's (Table 2), which was probably an effect of the conversion of microwave energy into thermal energy. However, because the intensity of microwaves from the Smartphone's fluctuated irregularly between 0 and 1 mW/cm^2, a precise specific absorption rate (SAR) could not be calculated. If the intensity of microwaves is 0.5 mW/cm^2 (average of 0 and 1) and assuming that 100% of the microwave energy can be converted into thermal energy, the SAR can be calculated to be approximately 1.5 W/kg for a 3 h exposure. This energy can increase the temperature by 1.6°C. Because the apparent increase in the OD_{630} value could be partially due to growth of the bacteria as a result of the elevated temperature, colorimetric data (OD_{630} value) was corrected to estimate the net SOS response by the ratio of the OD_{600} value (turbidity) of the receiver or transmitter side bacteria divided by that of the control bacteria.

As shown in Figure 5, we documented somewhat DNA damage in bacteria that were positioned next to Smartphone's, although differences were insignificant statistically between both samples placed next to the Smartphone's and the sham-exposed samples. In fact, at the receiving Smartphone's, only a 0.6℃ increase was observed, less than the 1.6°C calculated from the SAR, indicating that most thermal energy was removed by the temperature-controlling set-up, however, some contribution of the thermal effects was still presumable. The possible harmful effects detected here would include non-thermal effects as well as thermal effects because we normalized the raw colorimetric data by the ratio: (OD_{600} value of exposed control) / (OD_{600} value of sham-exposed control). Anyway, our simple assay is expected to be a suitable method for the detection of the effects of microwave irradiation.

Even when mobile phones are used infrequently or for short periods, the cumulative effect of emitted microwaves is a serious issue, particularly for juveniles, because tissue formation by cell division in newborns is more active compared with that in adults. Long-term exposure to microwaves, even low-emission-strength frequencies, may be harmful to human health. Further studies are warranted to estimate the long-term health implications of mobile phone use. The simple detection system described in the current study could also be used to assess the impact of equipment that is designed to protect people from the harmful effects of microwaves emitted from instruments including mobile phones.

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REFERENCES

[1] Hardell L, Mild KH, Carlberg M. Further aspects on cellular and cordless telephones and brain tumours, *Int. J. Oncol*. 2003; 22: 399–407.

[2] Hardell L, Carlberg M, Hansson MK. Use of cellular telephones and brain tumour risk in urban and rural areas, *Occup. Environ. Med.* 2005; 62: 390–339.

[3] Lehrer S, Green S, Stock RG. Association between number of cell phone contracts and brain tumor incidence in nineteen U.S. States, *J. Neurooncol*. 2011; 101: 505–507.

[4] Hardell L, Carlberg M, Hansson MK. Epidemiological evidence for an association between use of wireless phones and tumor diseases, *Pathophysiology*. 2009; 16: 113–122.

[5] Hardell L, Carlberg M, Hansson MK, Eriksson M. Case-control study on the use of mobile and cordless phones and the risk for malignant melanoma in the head and neck region, *Pathophysiology*. 2011; 18: 325–333.

[6] Singh K. Nagaraj A. Yousuf A. Ganta S. Pareek S. Vishnani P., Effect of electromagnetic radiations from mobile phone base stations on general health and salivary function, *J. Int. Soc. Prev. Community. Dent.* 2016; 6: 54–59.

[7] Warren HG, Prevatt AA, Daly KA, Antonelli PJ. Cellular telephone use and risk of intratemporal facial nerve tumor, *Laryngoscope*. 2003; 113: 663–667.

[8] Hardell L, Hallquist A, Hansson MK, Carlberg M, Gertzén H, Schildt EB, Dahlqvist A。 No association between the use of cellular or cordless telephones and salivary gland tumours, *Occup. Environ. Med.* 2004; 61: 675–679.

[9] Mortazavi SM, Mahbudi A, Atefi M, Bagheri S, Bahaedini N, Besharati A. An old issue and a new look: electromagnetic hypersensitivity caused by radiations emitted by GSM mobile phones, *Technol. Health. Care.* 2011; 19: 435–443.

[10] Hardell L, Hallquist A, Mild KH, Carlberg M, Pahlson A, Lilja A. Cellular and cordless telephones and the risk for brain tumours, *Eur. J. Cancer. Prev*. 2002; 11: 377–386.

[11] Leszczynski D, Joenvaara S, Reivinen J, Kuokka R. Non-thermal activation of the hsp27/p38MAPK stress pathway by mobile phone radiation in human endothelial cells: molecular mechanism for cancer- and blood-brain barrier-related effects, *Differentiation*. 2002; 70: 120–129.

[12] Mashevich M, Folkman D, Kesar A, Barbul A, Korenstein R, Jerby E, Avivi L. Exposure of human peripheral blood lymphocytes to electromagnetic fields associated with cellular phones leads to chromosomal instability, *Bioelectromagnetics*. 2003; 24: 82–90.

[13] Zotti-Martelli L, Peccatori M, Maggini V, Ballardin M, Barale R. Individual responsiveness to induction of micronuclei in human lymphocytes after exposure in vitro to 1800-MHz microwave radiation, *Mutat. Res*. 2005; 582: 42–52.

[14] Mortavazi S, Habib A, Ganj-Karami A, Samimi-Doost R, Pour-Abedi A, Babaie A. Alterations in TSH and thyroid hormones following mobile phone use, *Oman*. Med. J. 2009; 24: 274–278.

[15] Kwon MS, Vorobyev V, Kännälä S, Laine M, Rinne J, Toivonen T, Johansson J, Teräs M, Lindholm H, Alanko T, Hämäläinen H. GSM mobile phone radiation suppresses brain glucose metabolism, *J. Cereb. Blood. Flow. Metab*. 2011; 31: 2293–2301.

[16] Yakymenko I, Sidorik E, Kyrylenko S, Chekhun V. Long-term exposure to microwave radiation provokes cancer growth: evidences from radars and mobile communication systems, *Exp. Oncol.* 2011; 33: 62–70.

[17] Garaj-Vrhovac V, Orescanin V. Assessment of DNA sensitivity in peripheral blood leukocytes after occupational exposure to microwave radiation: the alkaline comet assay and chromatid breakage assay, *Cell. Biol. Toxicol*. 2009; 5: 33–43.

[18] Xu S, Chen G, Chen C, Sun C, Zhang D, Murbach M, Kuster N, Zeng Q, Xu Z. Cell type-dependent induction of DNA damage by 1800 MHz radiofrequency electromagnetic fields does not result in significant cellular dysfunctions, PLoS. One. 2013; 8: e54906.

[19] Ni S, Yu Y, Zhang Y, Wu W, Lai K, Yao K. Study of oxidative stress in human lens epithelial cells exposed to 1.8 GHz radiofrequency fields, PLoS. One. 2013; 8: e72370.

[20] Esmekaya MA, Seyhan N, Kayhan H, Tuysuz MZ, Kurşun AC, Yağcı M. Investigation of the effects of 2.1 GHz microwave radiation on mitochondrial membrane potential (ΔΨm), apoptotic activity and cell viability in human breast fibroblast cells, Cell. *Biochem. Biophys.* 2013; 67: 1371–1378.

[21] Haarala C, Bjornberg L, Ek M, Laine M, Revonsuo A, Koivisto M, Hamalainen H. Effect of a 902 MHz electromagnetic field emitted by mobile phones on human cognitive function: A replication study, *Bioelectromagnetics*. 2003; 24: 283–288.

[22] Zeni O, Romanò M, Perrotta A, Lioi MB, Barbieri R, d'Ambrosio G, Massa R, Scarfì MR. Evaluation of genotoxic effects in human peripheral blood leukocytes following an acute in vitro exposure to 900 MHz radiofrequency fields, *Bioelectromagnetics*. 2005; 26: 258–265.

[23] Chauhan V, Mariampillai A, Gajda GB, Thansandote A, McNamee JP. Analysis of proto-oncogene and heat-shock protein gene expression in human derived cell-lines exposed in vitro to an intermittent 1.9 GHz pulse-modulated radiofrequency field, *Int. J. Radiat. Biol.* 2006; 82: 347–354.

[24] Scarfì MR, Fresegna AM, Villani P, Pinto R, Marino C, Sarti M, Altavista P, Sannino A, Lovisolo GA. Exposure to radiofrequency radiation (900 MHz, GSM signal) does not affect micronucleus frequency and cell proliferation in human peripheral blood lymphocytes: an interlaboratory study, *Radiat. Res*. 2006; 165: 655–663.

[25] Tanaka Y, Matsuura Y, Tsubouti S, Nagaike T, Izumi M, Shimizu N. Studies on the effects of microwaves emitted from mobile phones on human physiology, *Eastern. Medicine. 2007; 23: 23–30.*

[26] Oftedal G, Straume A, Johnsson A, Stovner LJ. Mobile phone headache: a double blind, sham-controlled

provocation study, *Cephalalgia*. 2007; 27: 447–455.

[27] Zeni O, Schiavoni A, Perrotta A, Forigo D, Deplano M, Scarfi MR. Evaluation of genotoxic effects in human leukocytes after in vitro exposure to 1950 MHz UMTS radiofrequency field, *Bioelectromagnetics*. 2008; 29: 177–184.

[28] Mizuno Y, Moriguchi Y, Hikage T, Terao Y, Ohnishi T, Nojima T, Ugawa Y. Effects of W-CDMA 1950 MHz EMF emitted by mobile phones on regional cerebral blood flow in humans, *Bioelectromagnetics*. 2009; 30: 536–544.

[29] Hintzsche H, Stopper H. Micronucleus frequency in buccal mucosa cells of mobile phone users, *Toxicol. Lett*. 2011; 193: 124–130.

[30] Bourthoumieu S, Terro F, Leveque P, Collin A, Joubert V, Yardin C. Aneuploidy studies in human cells exposed in vitro to GSM-900 MHz radiofrequency radiation using FISH, *Int. J. Radiat. Biol.* 2011; 87: 400–408.

[31] Silva V, Hilly O, Strenov Y, Tzabari C, Hauptman Y, Feinmesser R. Effect of cell phone-like electromagnetic radiation on primary human thyroid cells, *Int. J. Radiat. Biol. 2016; 92: 107–115.*

[32] Dutta SK, Nelson WH, Blackman CF, Brusick DJ. Cellular effects in microbial tester strains caused by exposure to microwaves or elevated temperatures, *J. Environ. Pathol. Toxicol.* 1980; 3: 195-206.

[33] Filipic M, Umek A, Mlinaric A. Screening of Basidiomycete mushroom extracts for antigenotoxic and bio-antimutagenic activity, *Pharmazie*. 2002; 57: 416–420.

[34] Oda Y, Funasaka K, Kitano M, Nakama A, Yoshikura T. Use of a high-throughput *umu*-microplate test system for rapid detection of genotoxicity produced by mutagenic carcinogens and airborne particulate matter, *Environ. Mol. Mutagen*. 2004; 43: 10–19.

[35] Okuno Y, Miyazawa M. Suppressive components in rice husk against mutagens-induced SOS response using *Salmonella typhimurium* TA1535/pSK1002 *umu* test, *Nat. Prod. Res.* 2007; 21: 805–809.

[36] Hisama M, Matsuda S, Tanaka T, Shibayama H, Nomura M, Iwaki M. Suppression of mutagens-induced SOS response by phytoncide solution using *Salmonella typhimurium* TA1535/pSK1002 *umu* test, *J. Oleo*. Sci. 2008; 57: 381–390.

[37] Dawe AS, Smith B, Thomas DW, Greedy S, Vasic N, Gregory A, Loade RB, de Pomerai DI. A small temperature rise may contribute towards the apparent induction by microwaves of heat-shock gene expression in the nematode Caenorhabditis Elegans, *Bioelectromagnetics*. 2006; 27: 88–97.

Figures and Legends

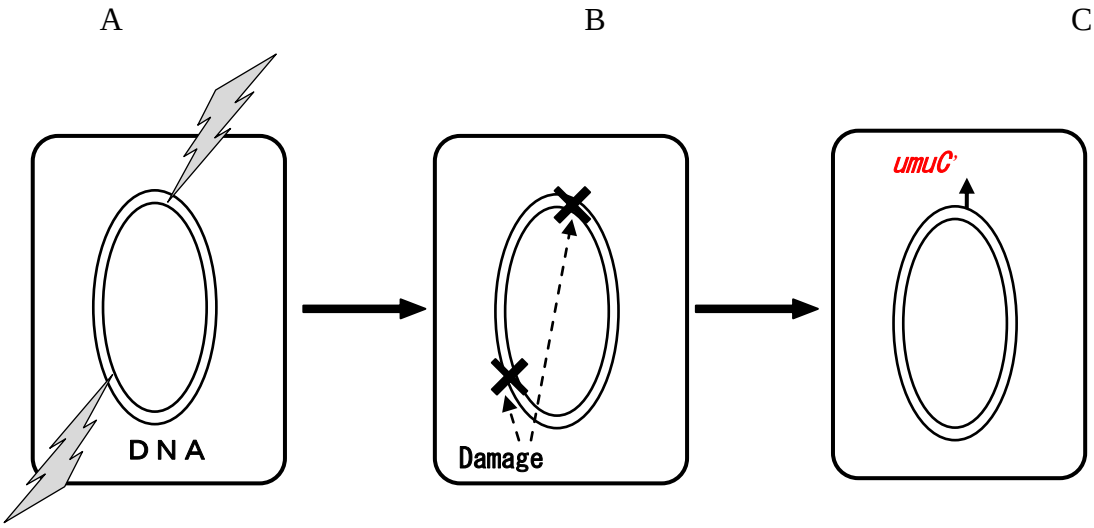


Figure 1. Schematic of the principle behind the *umu* test (modified from the web site of Iwaki Co. Ltd., http://www.pro-purify.co.jp/umulac_at/sokuteigenri.html). When damage is caused to DNA in the *Salmonella* cells (Figure 1A and 1B), the SOS reactions is initiated, resulting in the expression of the *umuC'* gene (Figure 1C).

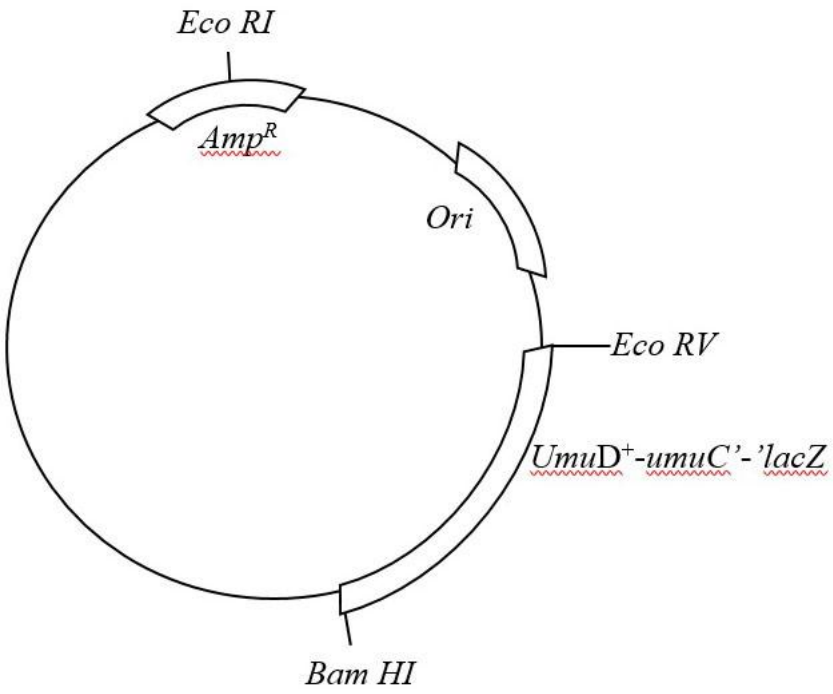
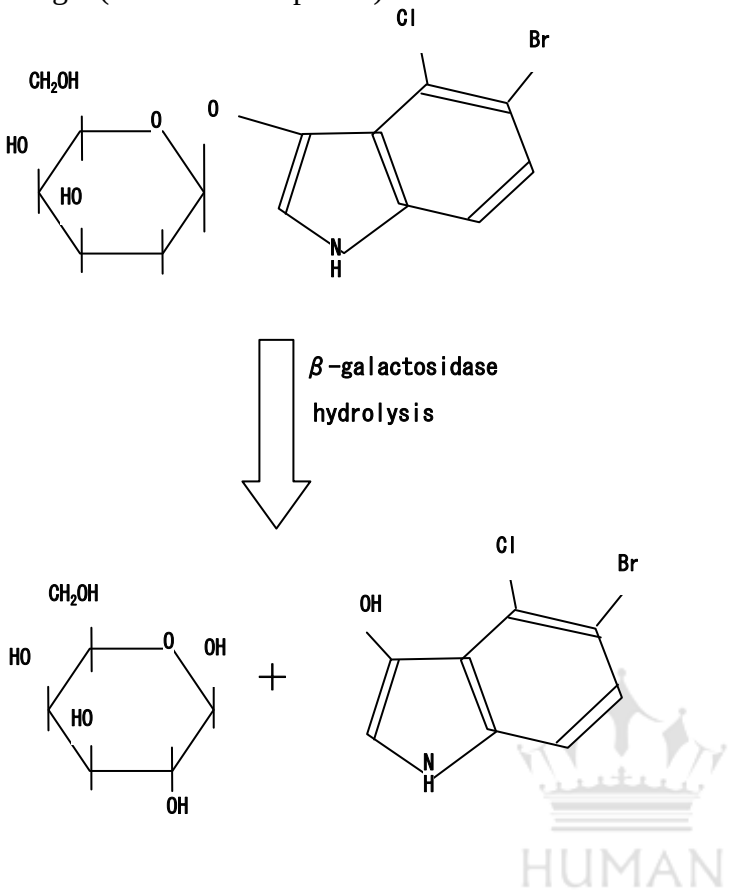


Figure 2. Construction of pSK1002 vector included in the NM2009 bacterial strain (drawn with reference to Oda *et al*. [34]. For this plasmid, the expression of the hybrid *umuD*$^{+}$*-umuC'-'lacZ* gene can be detected enzymatically. The enzyme *umuC'* is related to the error-prone steps of DNA repair.

Amp^{R}: ampicillin-resistance gene. *Eco RI*, *Eco RV*, *Bam HI*: restriction sites by those restriction enzymes. *Ori*: the origin of plasmid replication.

Figure 3.

X-gal (colorless transparent)



Sugar component (galactopyranose) Indole (blue color)

Figure 3. Principle of the measurement of *lac* activity. One of the reactions of the SOS response is the expression of the *umuC'* gene, followed by the expression of the *'lacZ*. This enzyme converts the colorless X-gal sugar component (galactopyranose) into the blue-colored indole. Thus, the intensity of the blue color increases depending on the genotoxicity of the mutagen.

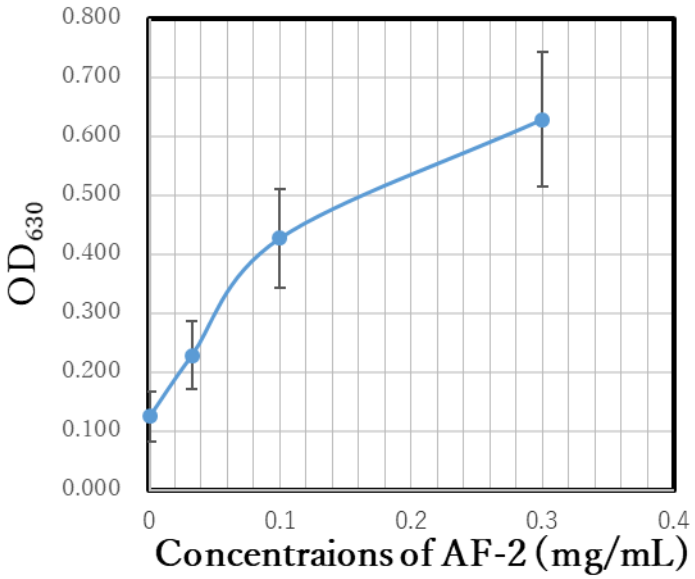


Figure 4. Average OD_{630} measured for various concentrations of AF-2. Error bars represent standard deviation (n= 3).

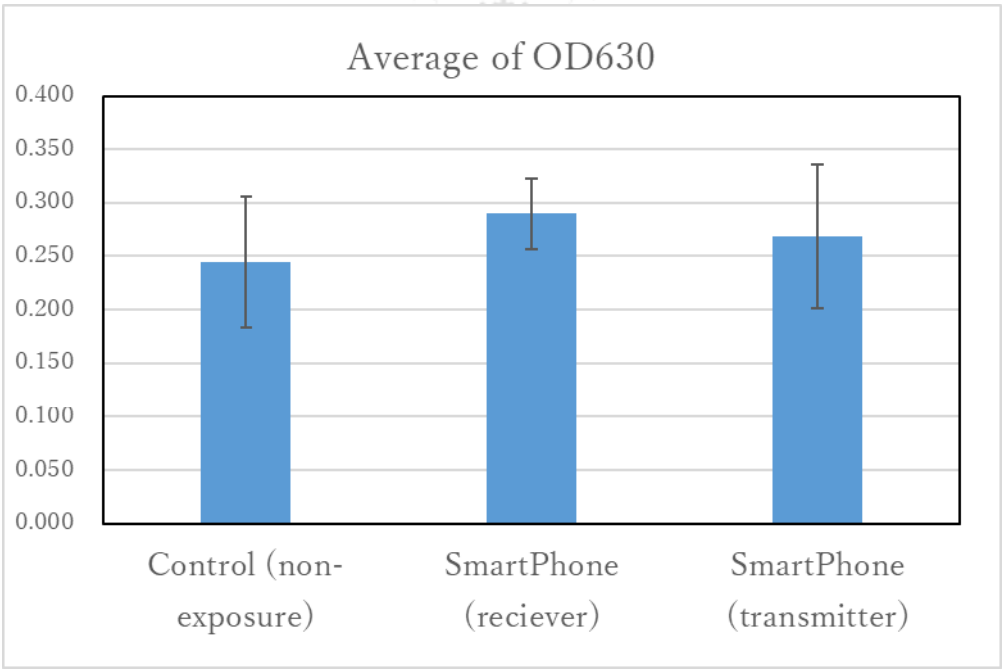


Figure 5. Average OD_{630} for the control and for the smartphone treatments (receiver side and transmitter side). Error bars represent standard deviation (n = 3).

Table 1. Intensity of the microwaves measured by a Trifield Meter.

Measurement location	Microwave intensity (mW/cm^2)
1 cm from the transmitting smartphone	fluctuated between 0 and 1 irregularly
1 cm from the receiving smartphone	fluctuated between 0 and 1 irregularly
4 m from the two smartphones (control)	0

Table 2. Average temperatures of the bacteria tubes.

Location	Average temperature (°C)
4 m from the smartphones (control)	25.3
receiving smartphone	25.8
transmitting smartphone	25.2