Effects of Irradiation on Microbial Community Structure in the Yangtze River and Selection of Representative Microorganisms

J B SONG[1] **and L P KUAI**[1]

1 School of Nuclear Science and Engineering, Shanghai Jiao Tong University，Shanghai 200240, China

243887682@qq.com , L P KUAI E-mail：sjtu-lkuai@qq.com;

Abstract. In the field of radioactive contamination, few studies were conducted on using microorganisms for biological monitoring. However, studies regard microorganisms as pollution indicator organisms are very common in the field of heavy metal and pesticide pollution. In this study, we chose the Yangtze River water as the research object, and studied the changes of species and quantity of cultivable bacteria in water samples under different irradiation by separation and purification methods. Results indicated that irradiation will cause the change of microbial community structure and the decline of their quantity; Among them, (flavobacterium) is mostly affected and (bacillus) is less affected. Finally, we found two representative microorganisms (Flavobacterium nitratireducens strain N1; Novosphingobium aquiterrae strain E-II-3) in the Yangtze Water and provided a reference for using microorganisms to monitor the radioactive pollution.

1. Introduction

At present, in order to ensure the required energy for economic development in China's inland region, the construction of inland nuclear power plants will become the inevitable demand [1]. Compared with coastal nuclear power plants, inland nuclear power plants' volume of the receiving water is less. Once the nuclear leakage accident happens, it will have great influence on local residents' production and life. Therefore, China has put forward strict requirements for inland nuclear power plants to achieve zero emission [2]. At the same time, inland nuclear power plants need to use more scientific monitoring standards to make a more objective evaluation [3]. Among them, biological monitoring can be used as an important means, which makes a more objective evaluation about the impact from radionuclides that are released from inland nuclear power plants under their normal operation, accident condition and retire process on the environment [4].

As the population which has the largest number, maximum biomass, and the biggest influence on the cycle of life elements in the nature, microbe has a unique advantage when used for monitoring water environment [5]. As the most basic member of the ecological system, microorganisms are more sensitive to pollution elements, such as heavy metals and organic matters in the environment. They can quickly feel a series of changes in the environment and make corresponding reactions through the related mechanism. Therefore, microorganism is considered as the most potential indicating organism [6].

Currently, in a series of fields polluted by heavy mental and pesticides, related researches choosing microorganisms as pollution indicating organisms have made remarkable achievements [7]. However, in the field of radioactive pollution, researching reports choosing microorganisms to do the biological monitoring are scarce. By comparing the changes of cultivable microorganisms before and after irradiation in the Yangtze River water sample, this paper researches the influence from irradiation on the structure of microbial population. Meanwhile, it looks for representative microorganisms in the water body and provides the corresponding reference for the biological monitoring of the radioactive pollution in Yangtze River basin.

2. Materials and Methods

2.1. Water Sample Collection

Referring to "Surface Water and Sewage Test Specification", we collected water samples in three points of Yangtze River area on April 15, 2016. The sampling location is shown in figure 1.

Figure.1 Location of sampling sites

We used organic glass sampler to collect the water sample under 0.5 m of the water surface, mixed the water sample, loaded it into plastic bottles disposed by moist heat sterilization in advance, put the sample in the heat preservation box, and immediately sent it to Shanghai Metrological Testing Technology Research Institute to do the irradiation.

2.2. Sample Irradiation

Sample Irradiation: Using 60Co to do the irradiation for the sample, with dose respectively 2 Gy、20 Gy、200 Gy and dose rate 4Gy/h, and taking a group sample without irradiation as the blank control.

2.3. Analysis Method

2.3.1. Counting bacteria in the sample. After being diluted to 10-1, 10-2, 10-3 and 10-4, the sample is coated on the agar plate and cultivated in 30℃ [8]. Everyday, we observe bacterial colonies' morphology and also do the community statistics. According to bacterial colonies' size, color, uplift situation and edge shape, we distinguish different types of strains.

2.3.2. *Single strain's physiological and biochemical function determination.* After transferring out the strain cultured from R2A table, we purify it three times. Referring to "Common Bacterial System Identification Manual" [9], we do the physiological and biochemical function experiment for single strain and preliminarily identify them.

2.3.3. *Single strain molecular biology identification.* Strain will be sent to Shanghai Personal Biotechnology Co., Ltd for DNA extraction, PCR amplification and sequence determination, and determine the strain.

2.3.4. Correlation analysis. Using software Origin Lab 9.0 to do the curve fitting about the total number of bacteria and the changing number of single strain under different irradiation doses, and understanding the correlation between the irradiation intensity and the changing number of the strain.

2.3.5. Community diversity analysis. Four community diversity indexes [10]: Community diversity abundance level (S), dominance level (P), Simpson index (L) and Shannon-Wiener index (H).

S: The number of different species of bacteria in the sample

P: The proportion of a certain type of bacteria,

$$p_i = n_i/N \quad (1)$$

L: Referring to the probability of the same type of bacteria selected from two colonies randomly. The smaller *L* indicates that the population distribution is more uniform and species are more abundant.

$$L = \sum_{i=1}^{S}[n_i(n_i-1)/N(N-1)] \quad (2)$$

H: The composite index that can reflect the community evenness and diversity. The larger *H* indicates that the biodiversity is higher.

$$H = -\sum_{i=1}^{S}\left(\frac{n_i}{N}\right)\ln(n_i/N) = -\sum_{i=1}^{S} p_i \ln p_i \quad (3)$$

3. Conclusion and analysis

3.1. Identification results

Eighteen kinds of bacterial colonies are separated from the water body and are named respectively as a-r. We can conduct physiological and biochemical experiments (Table 1) and molecular biological identification (Table 2) for all 18 kinds of bacterial colonies. The result shows that bacterial colonies from the separation belong to different species. Among them, the share of *Flavobacterium*, *Bacillus* and *Acinetobacter* is larger.

Table.1 Physiological and biochemical characteristics of strains

	a	b	c	d	e	f	g	h	i	j	k	l	m	n	o	p	q	r
Methyl test	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-
VP test	-	-	-	-	-	-	-	-	+	-	-	-	-	+	+	-	-	-
Starch hydrolysis	-	-	-	-	+	-	+	-	+	+	-	+	+	-	+	-	-	+
Gelatin liquefaction	-	-	+	+	-	-	-	+	+	-	+	+	+	+	+	-	+	-
H_2S production	+	+	+	+	+	-	+	+	+	+	+	+	-	+	+	+	+	+
Catalase test	+	-	+	+	-	+	+	+	+	+	-	+	+	-	+	-	+	-
Nitrate reduction	-	+	+	+	-	-	-	+	+	-	-	+	-	+	-	-	+	+
Acid from Glucose(open)	+	+	-	+	-	-	+	-	-	-	+	-	+	-	+	-	+	-
Gas from Glucose(open)	-	+	-	-	-	-	+	-	-	-	+	-	+	-	+	-	+	-

Table.2 Bacterial identification results

Bacteria	DNA identification results	Identities
a	*Rhodococcus equi strain DSM 20307*	99%

b	*Novosphingobium soli strain CC-TPE-1*	98%
c	*Flavobacterium glycines strain NBRC 105008*	97%
d	*Flavobacterium nitratireducens strain N1*	98%
e	*Hymenobacter ocellatus strain Myx 2105*	93%
f	*Acinetobacter johnsonii strain ATCC 17909*	99%
g	*Bacillus stratosphericus strain 41KF2a*	100%
h	*Nocardioides marinus strain CL-DD14*	98%
i	*Bacillus aryabhattai strain B8W22*	98%
j	*Empedobacter halobium strain G393-B445*	99%
k	*Novosphingobium aquiterrae strain E-II-3*	97%
l	*Flavobacterium pectinovorum strain DSM 6368*	97%
m	*Porphyrobacter colymbi strain TPW-24*	99%
n	*Flavobacterium plurextorum strain 1126-1H-08*	98%
o	*Exiguobacterium acetylicum strain DSM 20416*	99%
p	*Acinetobacter johnsonii strain DSM 6963*	97%
q	*Brevibacillus agri strain NBRC 15538 16S*	99%
r	*Pelomonas saccharophila strain DSM 654*	98%

3.2. Irradiation's effects on microbial quantity

After the irradiation, we count the number of various forms of bacterial colonies on the plate and get the bacterial community composition as shown in table 3 under different irradiation intensities. You can see that with the increase of irradiation dose, the number of microorganisms in the sample has a declining tendency. The number of bacteria in the sample decreases from 2.4X104 CFU/ml in the control group to 6.8X103 CFU/ml under 200 Gy irradiation intensity, total decreased by 71.67%, which shows that high irradiation intensity will make the inactivation of most microorganisms. A Krisko's [11] experiments also come to the similar conclusion. However, when the irradiation dose is 2 Gy, the total number of bacteria and the number of bacteria in the control group are both around 2.2X104 CFU/ml. Both are flat, showing that the irradiation dose won't have extremely large effects on microorganisms.

Table.3 The flora composition of bacteria in different irradiance sample

Bacteria	a	b	c	d	e	f	g	h	i	j	k	l	m	n	o	p	q	r	Total
Dose	Number of colony-forming units(10^2CFU/ml)																		
200Gy	4	3	7	11	5	13	6	1	7	1	4	2	3	0	0	0	0	1	68
20Gy	1	6	15	42	12	35	2	2	12	0	9	0	3	2	3	1	4	0	149
2Gy	0	9	42	85	15	30	0	0	8	0	19	0	4	0	4	0	5	0	221
0Gy	0	6	34	95	8	22	0	5	8	0	36	0	10	0	5	0	6	0	235

3.3. Irradiation's effects on microbial species

According to the table 3, the analysis of cultivable bacterial communities' structure before and after the irradiation in the Yangtze River water shows that: When the irradiation dose is 2 Gy, the structure of bacteria community hasn't changed. It indicates that the irradiation of this dose has very small effects on microorganisms, which is consistent with the influence from irradiation on the number of bacteria. With the increase in irradiation intensity, the number of bacterial species goes up 4 instead. Since the irradiation leads to the death of lots of sensitive dominant strains, other strains get sufficient growth space. Table 4 shows that Simpson index (*L*) decreases with the increase of irradiation, which proves the conclusion that when the irradiation dose increase, the bacterial communities will become more diversified and the community distribution will become more even. WS Elsayed's [12] studies also have the similar situations.

In the Yangtze River water, the proportion of *flavobacterium* is very high (over 50%), belonging to a dominant strain. With the increase in the irradiation intensity, the proportion of the dominant strain, *flavobacterium*, declines from 59.29% to 29.31%, the total number decreased by 84.50% , which is affected greatly from the irradiation. By contrast, the proportion of *Bacillus* in the control group is 3.35% and goes up to 19.12% in the 200Gy group, the total number increased by 38.46%, showing the strong irradiation tolerance of this strain.

Table.4 The community diversity index in different irradiance samples

Irradiation dose	Colony richness(*S*)	Dominant bacteria	Percentage(*P*)	Simpson index(*L*)	Shannon-Wiener index(*H*)
200Gy	14	*Flavobacterium*	29.31%	0.109	2.391
20Gy	15	*Flavobacterium*	38.93%	0.166	2.913
2Gy	10	*Flavobacterium*	57.62%	0.219	2.114
0Gy	11	*Flavobacterium*	58.29%	0.223	2.179

3.4. Irradiation's effects on specific strains

After observing figure 2, we can find that the changing tendency of *flavobacterium* dominant strain d （*flavobacterium nitratireducens strain N1*） is very close to the changing tendency of the number of strains in the figure 3 . And with the increase of the irradiation dose, its proportion is 40.43% in the control group and declines to 16.18% in the 200 Gy group. What's more, 88.42% of strain d （*flavobacterium nitratireducens strain N1*） has disappeared. The negative correlation between the number of strains and the irradiation dose successfully reflects the changing situation of microbial quantity when the irradiation intensity changes. It also shows that we can select this strain to study as the representative microorganism in the Yangtze River water.

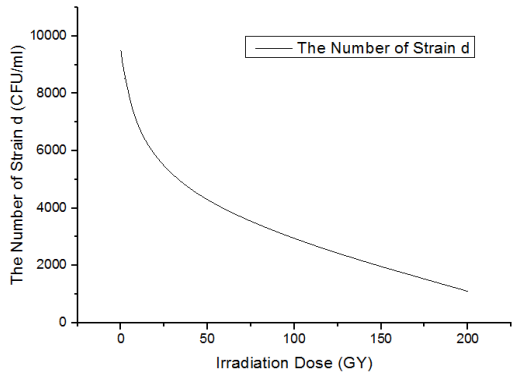

Figure.2 Variation of strain d under different irradiation doses

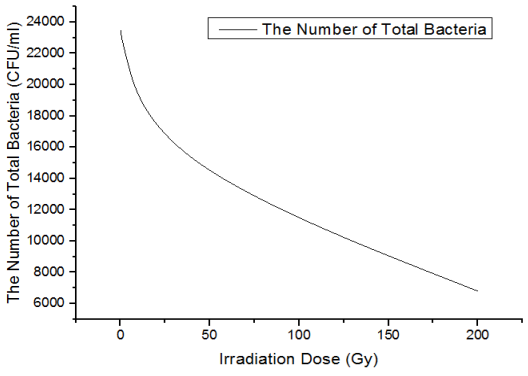

Figure.3 Variation of total bacteria under different irradiation doses

At the same time, as the Table 3 shows, the number of strain k（*novosphingobium aquiterrae strain E-II-3*）appears a declining tendency with the increase of the irradiation dose, which also can be study as a representative microorganism in the Yangtze River water.

4. Discussion

At present, most of domestic and overseas [13-14] studies about the influence of irradiation on microbes choose KGy dose. However, this experiment uses Gy magnitude to do the irradiation, where most microorganisms show a higher irradiation fatality rate. The main reason is that irradiation sample in this experiment is natural body, whose cultivable microbe concentration is only 2.35×10^4 CFU/ml. But other experiments choose the bacteria suspension experiencing the enrichment culture as the research object, whose cultivable microbe concentration is about 10^6—10^7 CFU/ml. Related researches [15] show that with a higher initial bacterium content, irradiation will have the smaller influence on microorganisms. In addition, this experiment chooses plastic containers. Compared with glass tubes used by M Barakatsm et al [14], plastic containers have a smaller density, whose atomic number is smaller and radiation permeability is better.

Damage for microorganisms from the irradiation has two types, one is the damage from materials, causing the disorder of enzyme system, physiological and biochemical delay or stop, and metabolic disorder or interrupt, and finally leading to the cell death. The second type of damage is from free radicals produced from the irradiation in the cytoplasm, leading to DNA single-stranded and double-stranded rupture and indirectly causing the death of microorganisms. Mainly through the rapid repair of damaged DNA fragments, microorganisms form spores to slow the metabolism rate and produce superoxide dismutase to remove free radicals this kind of ways to reduce the irradiation damage. *Bacillus* bacteria mentioned in this experiment can reduce moisture content through producing spores in adverse conditions and reduce metabolism rate to reduce irradiation damage, leading to a stronger irradiation tolerance. At the same time, Zhi Qixun [16] found that gram-positive bacteria have a higher radiation tolerance than gram-negative bacteria. Wujiang et al [17] came to the conclusion that the amount of superoxide dismutase in aerobic microorganism is higher than facultative anaerobic microorganism.

This paper selects d (*Flavobacterium nitratireducens strain N1*) and k (*Novosphingobium aquiterrae strain E-II-3*) which belong to facultative anaerobic non-spore gram-negative bacterium. Compared to other separated strains, they are more sensitive to the irradiation. Among them, strain d (*Flavobacterium nitratireducens strain N1*) belongs to the *Flavobacterium*. The result from Glockner F.O. et al [18] analysis of 16S rRNA in every country's water body shows that this kind of bacteria widely exists in the fresh water. Therefore, it has the researching value as the representative microorganism in the Yangtze River water.

Currently, related researches mainly focus on the influence from irradiation on the single strain in order to get irradiation resistant strain or find out the fatality rate caused by irradiation, such as LEENY et al [19] research about the irradiation lethal dose of Escherichia coli and bacillus cereus etc., and FA. Rainey et al [20] selection of irradiation resistant strain. This paper, however, focuses on the overall impact from irradiation on the cultivable microbes in the water body and explores the changing situation of microbe population's structure caused by irradiation. At the same time, this paper aims to look for the representative microorganism in the Yangtze River water and uses selected microorganism to provide the reference for radioactive pollution monitoring.

5. Conclusion

- Irradiation can lead to an increase in microbial community structure and the decline of its number in water body.
- Irradiation's influence on *Flavobacterium* is largest, but its influence on *Bacillus* is comparably smaller.
- Finding two kinds of representative microorganisms in the Yangtze river water, which are d (*Flavobacterium nitratireducens strain N1*) and k（*Novosphingobium aquiterrae strain E-II-*

3）, and choosing microorganisms to provide the reference for radioactive pollution monitoring in Yangtze River basin.

References

[1] Song M Q 2012 Analysis of the necessity of nuclear power development in inland China *Scientific and Technological Innovation and Application* **27** 178
[2] Guo X W, Tan B Q, Zhao J, 2013 Preliminary analysis of inland nuclear power for surface water quality impacts *Conservancy Technology and Economy* **19(4)** 5
[3] Beresford N A, Hosseini A, Brown J E 2010 Assessment of risk to wildlife from ionising radiation: can initial screening tiers be used with a high level of confidence *Journal of Radiological Protection* **30(2)** 265
[4] Copplestone D, Beresford N A, Brown J E 2013 An internationaldatabase of radionuclide concentration ratios for wildlife: development and uses *Journal of Environmental Radioactivity* **126(4)** 288
[5] Azarbad H, Niklińska M, CAM Van Gestel 2013 Microbial community structure and functioning along metal pollution gradients *Environmental Toxicology &Chemistry* **32(9)** 1992
[6] Ibekwe A M, Grieve C M 2004 Changes in developing plant microbial community structure as affected by contaminated water *Fems Microbiology Ecology* **48(2)** 239
[7] Zheng Y N, Wang S Y, Guo J H 2008 Trends in the use of protozoan and metazoan in the indication and assessment of WWTP operation *Microbiology* **35(12)** 1943
[8] Zhao B, Lin H, He S J 2014 *Microbiology Experiment (Beijing)* vol 2 (Science Press) p 58
[9] Dong X Z, Cai M Y 2001 *Systematic Determinative Manual of General Bacteria* (Beijing: Science Press) p 223
[10] Edwards L, Lilley A K, Timms-Wilson T H 2001 Characterisation of the culturable heterotrophic bacterial community in a small eutrophic lake *FEMS Microbiology Ecology* **35(3)** 295
[11] Krisko A, Radman M 2010 *Proceedings of the National Academy of Sciences of the United States of America* **107(32)** 14373
[12] Elsayed W S, Ghanem S 2009 Bacterial community structure change induced by gamma irradiation in hydrocarbon contaminated and uncontaminated soils revealed by PCR-denaturing gradient gel electrophoresis *Biotechnology* **8(1)** 78
[13] Qiu Y X, Ye S Y, Yu K 2007 Research on the lethal reason of radiation to Escherichia coli and Lactobacillus *Food Science and Technology* **6** 38
[14] Barakatsm M, Gary B, Richardh L 2010 Inactivation of Escherichia coli O157: H7, Listeria monocytogenes, Salmonella enterica and Shigella flexneri on Spinach Leaves by X-ray *Food Microbiology* **27(1)** 24
[15] Wei M, Yang P H, Li Q 2010 Main factors affecting sterilization effect of food irradiation *Science and Technology of Sichuan Agriculture* **3** 54
[16] Zhi Q Y 2010 *New Food Sterilization Technology* (Beijing: Agricultural Press) p 167
[17] Wu J, Chen D J 1997 Determination of SOD activity in microorganisms *Chinese Journal of Pharmaceuticals* **11** 495
[18] Frank Oliver Glöckner, E Zaichikov 2000 Comparative 16S rRNA Analysis of Lake Bacterioplankton Reveals Globally Distributed Phylogenetic Clusters Including an Abundant Group of Actinobacteria *Applied & Environmental Microbiology* **66(11)** 5053
[19] LEE N Y, JO C, SHIN D H 2006 Effect of γ-irradiation on Pathogens Inoculated into Ready-to-use Vegetables *Food Microbiology* **23(7)** 649
[20] Rainey F A, Ray K, Ferreira M 2005 Extensive diversity of Ionizing-Radiation-Resistant bacteria recovered from Sonoran desert soil and description of nine new species of the genus deinococcus obtained from a single soil Sample *Applied & Environmental Microbiology* **(71)9 5525**