

### III. Carriers for Biofertilizers

#### 1. Carrier Materials

##### 1.1. Introduction

Biofertilizers are usually prepared as carrier-based inoculants containing effective microorganisms. Incorporation of microorganisms in carrier material enables easy-handling, long-term storage and high effectiveness of biofertilizers. Among various types of biofertilizers, bacterial inoculant is one major group which includes rhizobia, nitrogen-fixing rhizobacteria, plant growth-promoting rhizobacteria, phosphate-solubilizing bacteria, and so on. Basically, the carrier-based inoculant of these bacteria can be prepared by a common procedure. In this chapter, type of carrier materials available for biofertilizers, and preparation in general of carrier-based inoculants will be described.

Most of the bacteria included in biofertilizer have close relationship with plant roots. *Rhizobium* has symbiotic interaction with legume roots, and rhizobacteria inhabit on root surface or in rhizosphere soil. To achieve the successful inoculation of *Rhizobium* or rhizobacteria, large population of the bacterial strain must be placed close to the emerging root, so that the majority of nodules are formed by the inoculated rhizobial strain, and that the inoculated rhizobacterial strain occupies the rhizosphere as major member of rhizobacteria. If the population is not large enough, the native rhizobia / rhizobacteria will occupy most of the root nodules / rhizosphere, leading to unsatisfactory effect of inoculation.

The most common way of inoculation is “seed inoculation”, in which the inoculant (bacteria-carrier mixture) is mixed with water to make slurry-form, and then mixed with seeds. In this case, the carrier must be a form of fine powder. To achieve the tight coating of inoculant on seed surface, use of adhesive, such as gum arabic, methylethylcellulose, sucrose solutions, and vegetable oils, is recommended. Any locally available sticky material, which is non-toxic to bacteria and seeds, can be used as adhesive.

Seed inoculation may not always be successful, i.e. the inoculation resulted in low nodule occupancy of the inoculated rhizobial strain, or low establishment of the inoculated rhizobacterial strain. This might be due to low population and/or low survival of the inoculated bacterial strain on the seed surface and in the soil. In such instance, “soil inoculation” will be adopted, whereby a large population of a bacterial strain can be introduced into the soil. For soil inoculation in general, granular inoculant is placed into the furrow under or alongside the seed. This enhances the chance for the inoculated strain to be in contact with plant roots.

##### 1.2. Carrier material

Various types of material are used as carrier for seed or soil inoculation. For preparation of seed inoculant, the carrier material is milled to fine powder with particle size of 10 -40  $\mu\text{m}$ . According to the “Handbook for Rhizobia” (Somasegaran and Hoben, Springer, 1994), the properties of a good carrier material for seed inoculation are: (1) non-toxic to inoculant bacterial strain, (2) good moisture absorption capacity, (3) easy to process and free of lump-forming materials, (4) easy to sterilize by autoclaving or gamma-irradiation, (5) available in adequate amounts, (6) inexpensive, (7) good adhesion to seeds, and (8) good pH buffering capacity. Needless to say, (9) non-toxic to plant, is another important property.

Peat is the most frequently used carrier material for seed inoculation. Peat-based rhizobial inoculant is already used in many countries and a number of information is available on the properties and effect of the inoculant.

For soil inoculation, carrier material with granular form (0.5 – 1.5 mm) is generally used. Granular forms of peat, perlite, charcoal or soil aggregates are suitable for soil inoculation. Various types of material used or tested as carrier for bacterial inoculant (mostly *Rhizobia*) is listed in Table 1.

Other essential criteria for carrier selection relating to survival of the inoculant bacteria should be considered. (1) Survival of the inoculant bacteria on seed. Seeds are not always sown immediately after seed coating with the inoculant bacteria. The bacteria have to survive on seed surface against drying condition until placed into soil. (2) Survival of the inoculant bacteria during the storage period. (3) Survival of the inoculant bacteria in soil. After being introduced into the soil, the inoculant bacteria have to compete with native soil microorganisms for the nutrient and habitable niche, and have to survive against grazing protozoa. Such carrier materials that offer the available nutrient and/or habitable micro-pore to the inoculant bacteria will be desirable. In this sense, materials with micro-porous structure, such as soil aggregate and charcoal, will be good carrier for soil inoculant.

### 1.3. Sterilization

Sterilization of carrier material is essential to keep high number of inoculant bacteria on carrier for long storage period.

Gamma-irradiation is the most suitable way of carrier sterilization, because the sterilization process makes almost no change in physical and chemical properties of the material. Detail of gamma-irradiation will be described in another chapter. In brief, carrier material is packed in thin-walled polyethylene bag, and then gamma-irradiated at 50 kGy (5 Mrads).

Another way of carrier sterilization is autoclaving. Carrier material is packed in partially opened, thin-walled polypropylene bags and autoclaved for 60 min at 121 °C. It should be noted that during autoclaving, some materials change their properties and produce toxic substance to some bacterial strains.

### 1.4. Appendix

As an example of the manipulation of rhizobial inoculant, our paper entitled “Enhanced Growth and Nodule Occupancy of Red Kidney Bean and Soybean Inoculated with Soil Aggregate-Based Inoculant” printed in *Soil Science and Plant Nutrition* (48 (2), 251-259, 2002) will be useful. Abstract of the paper is as follows. For the reprint request, please mail to [asenoo@mail.ecc.u-tokyo.ac.jp](mailto:asenoo@mail.ecc.u-tokyo.ac.jp).

#### ABSTRACT

Volcanic ash soil, which is widely distributed in Japan, contains a large amount of well-structured soil aggregates. By using these aggregates as carrier materials, we prepared (brady)rhizobial inoculants for red kidney bean (*Phaseolus vulgaris*) and soybean (*Glycine max*). Autoclaved soil aggregates were inoculated with *Rhizobium tropici* CIAT899R or *Bradyrhizobium japonicum* USDA110R, incubated for 15 or 21 days at 30°C, slowly air-dried at 20°C to prepare the aggregate-based inoculants, and stored at various temperatures. The populations of CIAT899R and USDA110R in the aggregate-based inoculants were maintained during several months of storage at 20°C. When the aggregate-based inoculants were mixed with soil, CIAT899R and USDA110R cells showed a remarkably improved survival in soils compared with those mixed with soil without carrier material. The effect of the aggregate-based inoculants on the growth of red kidney bean and soybean was examined in pot experiments. By placing a small amount of the inoculant just beneath the seeds at the time of sowing, plant growth was significantly enhanced compared with the use of traditional peat-based inoculant. In addition, nodule formation on the upper part of soybean roots and nodule occupancy by the inoculated strain were remarkably enhanced by the aggregate-based inoculant. It is suggested that soil aggregates might be suitable carrier materials for preparing cheap and effective (brady)rhizobial inoculants.

Table 1 Carriers materials used for biofertilizers

Carrier material	Inoculant bacterium	Characteristics
Sterilized oxalic acid industrial waste <sup>1</sup>	<i>Rhizobium</i>	<ul style="list-style-type: none"> <li>- seed inoculation</li> <li>- <i>Rhizobium</i> multiplication in carrier in ambient temperature up to 90 days.</li> <li>- Carrier sterilization contributed significant increase in grain yield, nodule number and nitrogen content.</li> </ul>
Alginate-perlite dry ganule <sup>2</sup>	<i>Rhizobium</i>	<ul style="list-style-type: none"> <li>- soil inoculation</li> <li>- <i>Rhizobium</i> strains survived in dry granules beyond 180 days.</li> <li>- The inoculant can be stored in a dry state without losing much viability.</li> </ul>
Composted sawdust <sup>3</sup>	<i>Bradyrhizobium</i> , <i>Rhizobium</i> and <i>Azospirillum</i>	<ul style="list-style-type: none"> <li>- seed inoculation</li> <li>- Good growth and survival of the inoculant strains.</li> </ul>
Agriperlite, Expanded clay, Kaolin, Celite, Diatom, Porosil MP, Micro-cel, Vermiculite <sup>4</sup>	<i>Agrobacterium radiobacter</i> K84	<ul style="list-style-type: none"> <li>- Crown gall control</li> <li>- Screening was performed to find improved formulation of K84 cells.</li> <li>- Effect of carrier storage temperature and carrier water content on survival of K84 was examined.</li> </ul>
Cheese whey grown cells in peat <sup>5</sup>	<i>Rhizobium meliloti</i>	<ul style="list-style-type: none"> <li>- seed inoculation</li> <li>- Better survival at various temperature during storage, even under desiccation</li> </ul>
Mineral soils <sup>6</sup>	<i>Rhizobium</i>	<ul style="list-style-type: none"> <li>- seed inoculant</li> <li>- <i>Rhizobium</i> survived better at 4 C than at higher temperature.</li> </ul>
Coal <sup>7</sup>	<i>Rhizobium</i>	<ul style="list-style-type: none"> <li>- seed inoculant</li> <li>- Seven among eight tested coals supported the growth and survival of <i>R. phaseoli</i> strains. Most contained more than 10<sup>7</sup> rhizobia per g after 12months.</li> </ul>
Granular inoculants amended with nutrients <sup>8</sup>	<i>Bradyrhizobium japonicum</i>	<ul style="list-style-type: none"> <li>- soil inoculant</li> <li>- Betonite granules, illite and smectite granules, or silica granules amended with glycerol, Na glutamate and inoculated with either peat or liquid <i>Bradyrhizobium japonicum</i> inoculants.</li> <li>- enhanced early nodulation of soybean and increased N content of grain</li> </ul>
Soybean oil or peanut oil added with lyophilized cells <sup>9</sup>	<i>Rhizobium</i>	<ul style="list-style-type: none"> <li>- seed inoculant</li> <li>- Provide more protection than peat-based inoculant when rhizobia are inoculated on seeds and exposed to condition of drought and high temperature.</li> </ul>
Perlite <sup>10</sup>	<i>Rhizobium</i> , <i>Bradyrhizobium</i> , <i>Bacillus</i>	<ul style="list-style-type: none"> <li>- seed inoculant</li> <li>- Combination of a sucrose adhesive with the perlite carrier gave better survival of bacteria on seeds</li> <li>- Produced similar number of nodules, nodule dry</li> </ul>

		weight, crop yield and nitrogen content as peat-based inoculants
Wastewater sludge <sup>11</sup>	<i>Sinorhizobium meliloti</i>	- seed inoculant - Result showed the suitability of using sludge as a carrier because it had the same or a higher potential than peat to support survival of <i>S. meliloti</i> .
Wheat bran, sugarcane baggas <sup>12</sup>	<i>Rhizobium/ Bradyrhizobium</i> and rock-phosphate-solubilizing fungus <i>Aspergillus niger</i>	- soil inoculant - The number of codcultured microorganisms was the highest with peat, followed by bran and sugarcane baggas.
Nutrient-supplemented pumice <sup>13</sup>	<i>Rhizobium</i>	- seed inoculant - Good storage and handling properties and could be mixed directly with the seeds during the sowing process

## 2. Carrier Sterilization using $\gamma$ -irradiation

### 2.1. Introduction

About 100 years ago, an England scientist Ernest Rutherford designated three kinds of radiation release from uranium  $\alpha$ -ray,  $\beta$ -ray and  $\gamma$ -ray. The entities of  $\alpha$ -ray,  $\beta$ -ray and  $\gamma$ -rays are helium ions (positively charged particles), electrons (negatively charged particles) and photons (ionizing electromagnetic waves), respectively. These are collectively called “ionizing radiation”. For radiation sterilization purpose,  $\gamma$ -irradiation is the most suitable because of its high penetrating activity. In this chapter, the properties of ionizing radiation, the effects of radiation on microorganisms, the necessity of radiation sterilization as well as the practical example for carrier sterilization will be described.

### 2.2. The properties of ionizing radiation

Atoms are electrically neutral in that the number of negatively charged electrons is exactly equal to the number of positively charged protons. However, when there are energy sources available, atoms can gain or loss electrons and acquire a net electrical charge. This process is called “ionization”. In a simple term, ionization is the gain or loss of electrons. Ionization of atoms by  $\gamma$ -rays mainly proceeds through Compton effect. In this process,  $\gamma$ -ray collides with and transfers part of its energy to a loosely bound electron in an atom. The  $\gamma$ -ray with reduced energy is scattered in a new direction and involved in the ionization of other atoms until it loses energy enough for ionization reaction. As a result of the  $\gamma$ -ray collision with an atom, an electron is ejected from its atom, and acts as  $\beta$ -ray (negatively charged particle) to create a new ionization that is mainly occurred by inelastic collision.

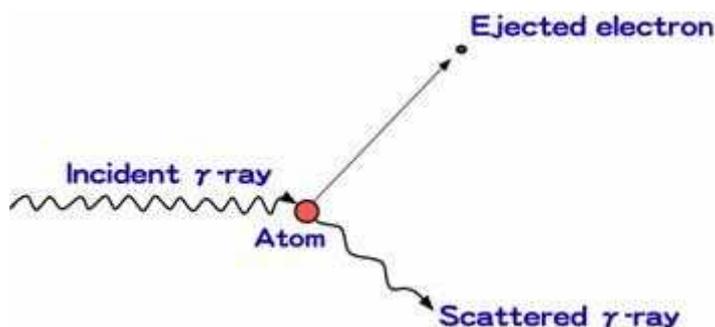


Fig. 1: Compton effect

### 2.3. The effects of radiation on microorganisms

If  $\gamma$ -rays collide with atoms of biological materials such as protein, lipid, carbohydrates and nucleic acid, the atoms are ionized and receive damages (direct effect of radiation). Among these biological materials, nucleic acid is the most sensitive material to ionizing radiation despite the proportion of nucleic acid in the total cellular components is only 1%.

From the aspect of biological effect of radiation, another important property of  $\gamma$ -rays is water radiolysis. The water molecule occupies 80% of the total cellular components. When a water molecule is ionized by  $\gamma$ -irradiation, many radical species including hydroxy radical, hydrogen radical, hydroperoxy radical and superoxide are produced. These radical species are highly reactive to biological materials, especially to nucleic acids. The effect of radiation on biological materials via water radiolysis is called “indirect effect of radiation”.

In general, there exist two types of DNA damage induced by the direct and indirect effects of radiation; DNA strand breaks and base oxidative damages. DNA strand break is the dissociation of the phosphodiester bond of the main chain in DNA. DNA strand break causes loss of the continuity of genetic information and arrest of replication process, thereby results in cell death. Base oxidative damage is occurred at the nucleotide base in DNA, and at the base moiety of nucleotide pool in cytosol. This type of DNA damage causes replication errors and the accumulation of genetic mutation, thereby results in cell death.

However, the radiation resistance of living organisms differs widely in individual species. In general, microorganisms exhibit more radiation resistance than animals and plants. One of the explainable reasons for this resistance is that the cell nucleus (target of radiation) of microorganisms is much smaller than those of animals and plants. Another reason can be explained by DNA protection and repair capacity. DNA protection includes spore formation (the resting stage of cell) and radical scavengers such as catalase, superoxide dismutase and carotenoids. Microorganisms have a great variety of DNA repair capacity with different effectiveness by which the difference in radiation resistance arises. It has been known that there exist non-spore-forming but extremely radiation resistant bacteria that inhabit in many natural places such as soil and environmental waste including animal dung and plant chip. However, all the radiation resistant bacteria isolated so far are non-pathogenic. The most problematic issue for the sterilization of microorganisms is the presence of soil-born spore-forming bacteria, which are highly resistant to radiation, desiccation and heat. Some spore-forming bacteria are infectious and highly pathogenic for human and farm animals; e.g. *Bacillus anthracis* (anthrax), *Clostridium tetani* (tetanus), *Clostridium botulinum* (gas gangrenous). Some other spore-forming bacteria with lesser toxicity can cause food poisoning and opportunistic infections.

As mentioned above, bacterial spore is highly resistant to radiation. However, when there are nutrients available, the spore germinates to produce a vegetative cell that is much more sensitive to radiation (Fig. 2). This process (germination) is occurred in minutes. The difference in the moisture condition can affect the radiation resistance. As shown in Fig. 3, wet cells of *Escherichia coli*, that are nonspore-forming bacterium, are more sensitive to radiation compared to desiccated cells. This difference in survival rate is due to indirect effect of radiation by water radiolysis. Therefore, controlling the moisture conditions is very important for effective sterilization of carrier materials.

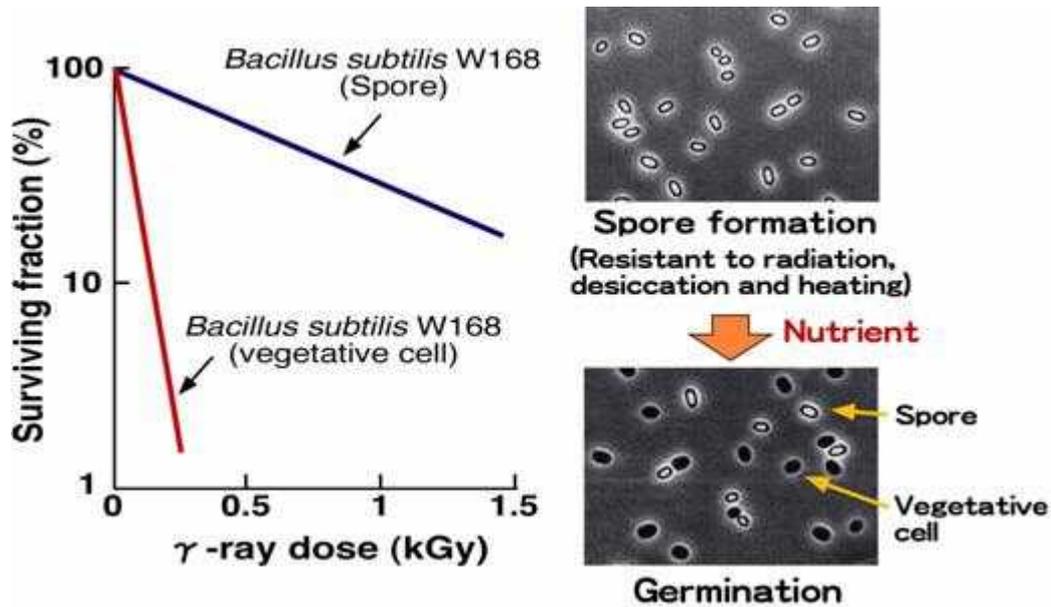


Fig. 2: Bacterial spore and vegetative cell

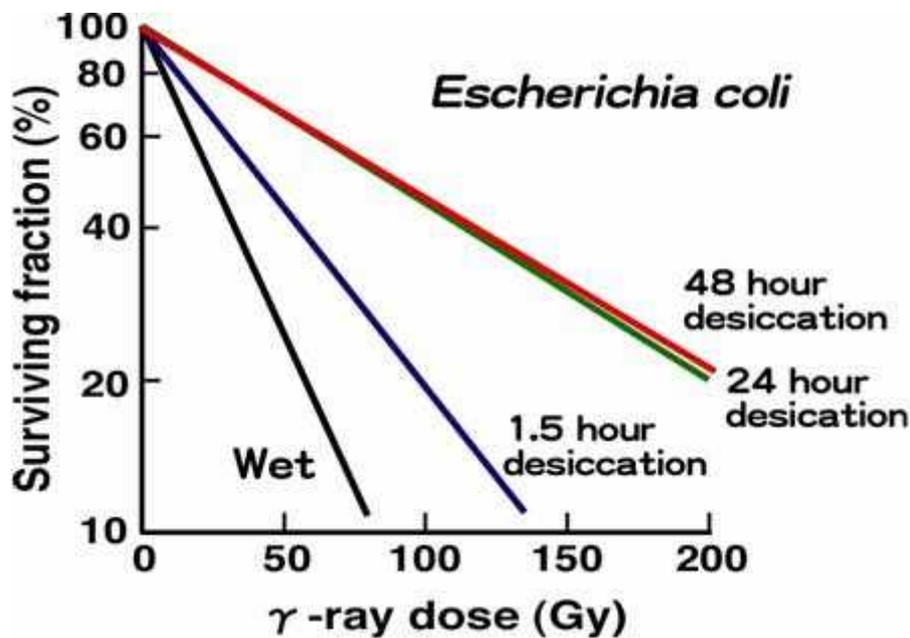


Fig. 3: Desiccation and hydration

#### 2.4. The necessity of radiation sterilization

The purpose of sterilization of carrier materials for biofertilizer can be divided into two categories. 1) To offer nutrient and place to the inoculant bacteria against the occupation by the contaminated and/or native bacteria. This is important to keep the number of inoculant bacteria on carrier during the storage period before use. 2) To prevent undesirable dispersion of pathogenic bacteria to agricultural field. In other words, radiation sterilization is essential to reduce the risk of field contamination and infection.

#### 2.5. The practical example for carrier sterilization

A proposed outline of sterilization process is described below.

##### a. Preparation of materials

a-1. Prepare the appropriate amount of carrier material (10 kg is recommended).

a-2. Divide into 10 polyethylene packages (Thickness: approx. 0.1 mm, Size: approx. 20 cm x 30 cm)

with 1 kg carrier.

a-3. Seal the packages using a heat sealer.

a-4. (Option 1) If the carrier is a highly dry material, wet with an appropriate amount of water (to increase the indirect effect of radiation).

a-5. (Option 2) If the presence of spore-forming bacteria is suspected in the carrier, add an appropriate amount of nutrient liquid medium (to promote the germination of spore).

b. Irradiation

b-1. Divide the carrier packages into 2 dose groups.

b-2. Irradiate each group by 25 kGy or 50 kGy of  $\gamma$ -rays at room temperature in the atmosphere. In the almost all cases, radiation sources are cobalt-60 or cesium-137. Irradiation dose can be controlled by changing the distance from the radiation source. The total irradiation time is dependent on the source activity. (Option: Instead of  $\gamma$ -rays, electron-beams can be used for radiation sterilization). A margin of error of plus or minus 10% is allowed for irradiation dose. No limit for dose rate. A short interruption of irradiation during the total time for required dose can be allowed. Follow the requirements for each irradiation facility. A practical example of irradiation is illustrated in Fig. 4.

b-3. After irradiation, preserve the irradiated packages at room temperature under the sealed condition until the inoculation of microorganisms.

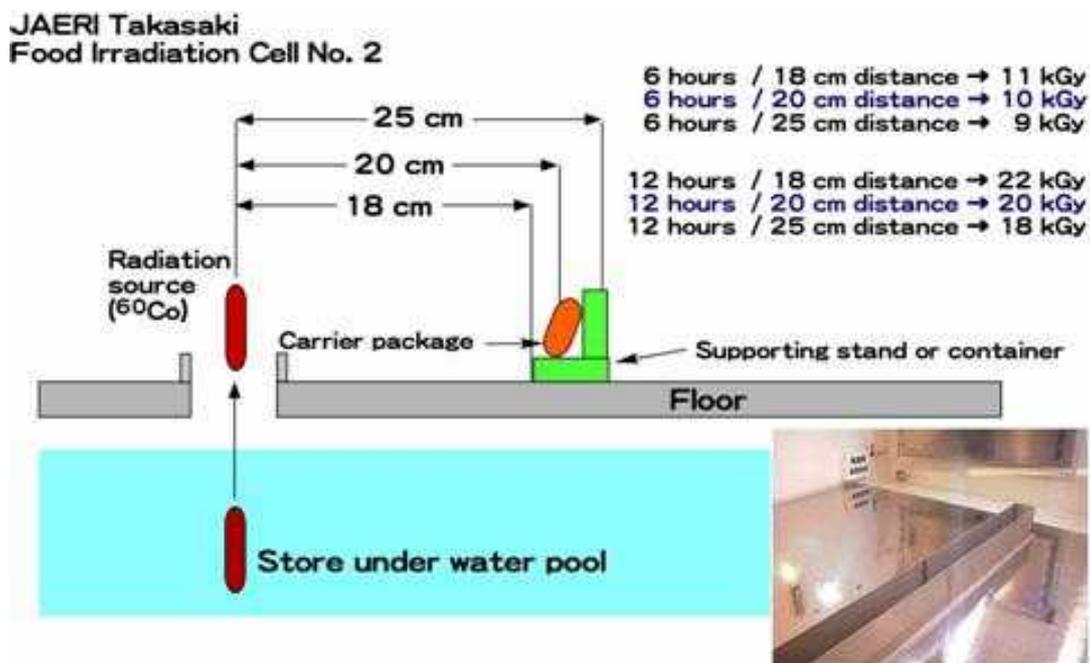


Fig. 4: A practical example of irradiation

d. Confirmation of sterilization effect.

d-1. Prepare 1 g of carrier samples (non-irradiated, 25 kGy and 50 kGy irradiated samples).

d-2. Mix with 9 ml of sterile water to make suspension.

d-3. Dilute the suspension by serial 10-fold dilutions using sterile water and spread on nutrient agar plates.

d-4. Incubate (at 30 °C in general) and count bacterial colony number.

(Note: For this experiment, some experimental equipment is required; autoclave, clean bench, temperature-controlled incubator, etc. The same protocol can be used for monitoring survival of the inoculant microorganisms in carrier during the storage period.)

- e. Inoculation of microorganisms to carrier.
- e-1. Prepare starter culture for inoculation. Optionally, appropriately dilute with sterile water for moisture and cell number adjustment.
  - e-2. Inject the culture to the carrier package using a sterile disposable plastic syringe with a needle.
  - e-3. Seal the needle hole with a waterproof tape.
  - e-4. Keep the package at appropriate temperatures for maturation and storage. Although the temperatures suitable for maturation and storage are dependent on the inoculant microorganisms, 30 °C for maturation and 20 °C-30 °C for storage will be suited for inoculants in most cases.

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## 2.6. Appendix:

$\gamma$ -ray irradiation facilities and electron-beam irradiation facilities potentially available for carrier sterilization in Asia are listed below.

### $\gamma$ -ray Irradiation Facilities for Commercial Use

Name	Year established	Shielding capacity	Source Activity	Purpose of irradiation	Remarks
<b>INDONESIA</b>					
1. Panoramic Irradiator	1979	125 kCi	75 kCi	Polymerization, Sterilization, Food Preservation	PATIR-BATAN
2. Latex irradiation	1984	400 kCi	215 kCi	Latex Vulcanization, Sterilization, Food Preservation	PATIR-BATAN
3. Indo Gamma	1991		4 MCi	Sterilization, Food Preservation	
4. Gamma Chamber	1998		10 kCi	Tissue Bank	Jamil Hospital
<b>KOREA</b>					
KAERI	1975 (1998)	176 cm	0.13 MCi	Research	Co60
Greenpia Tech	1986	~ 180 cm	1 MCi	Commercial	Co60
<b>MALAYSIA</b>					
Ansell	1977	4 MCi	2.0 MCi	sterilization	medical products
MINT	1989	2 MCi	1.5 MCi	sterilization	medical products/spices
Sterilgamma	1993	6 MCi	2.0 MCi	sterilization	medical products
ISOTRON	2001	4 MCi	1.0 MCi	sterilization	Medical products
<b>PHILIPPINES</b>					
Multipurpose irradiation facility, PNRI	1989	250,000 Ci	70,000 Ci	radiation sterilization; food irradiation	semi-commercial (pilot scale) service
<b>THAILAND</b>					
Kendal Gammatron Co.Ltd.	1984	500 kCi	150 kCi	Sterilization of medical supplies	Nakorn Prathom
Thai Irradiation Center	1993	3 MCi	450 kCi	R&D on radiation processing	Government own (OAP) Pratumthani
IBA S&I (Thailand) Ltd.	1999	3 MCi	1 MCi	Sterilization and others	Rayong
GAMMA-STER (Thailand) Ltd.	2000	6 MCi	1 MCi	Sterilization and others	Chonburi
Name	Year established	Shielding capacity	Source Activity	Purpose of irradiation	Remarks
<b>VIETNAM</b>					
SVST-Co-60	1999	2 MCi	400 kCi	- Sterilization of Medical products - Food pasteurization - Polysaccharides degradation	Hungarian type
RPP-150	1991	1 MCi	107 kCi	Food preservation	Russian type
Gamma Cell	1983 1987	16.5 kCi + 9 kCi		R&D (Present activity: 3 kCi)	Russian type

	Year established	Shielding capacity	Source Activity	Purpose of irradiation	Remarks
<b>JAPAN</b>					
Radia Ind. Co. Ltd.	1972		1.5 + 2 + 3 MCi	Sterilization	
Shihoro Agriculture Coop.	1973		1 MCi	Potato Irradiation	
Terumo Co. Ltd.	1983		3 × 2 MCi	Sterilization	
Koka Isotope Co. Ltd.	1987		2 MCi	Sterilization	
JMS Co. Ltd.	1987		3 × 2 MCi	Sterilization	
Nissho Co. Ltd.	1988		3 MCi	Sterilization	
Asahi-Medical Co. Ltd.	1988		1.5 MCi	Sterilization	
Japan Radiat. Serv. Co. Ltd.	1996		3 MCi	Sterilization	
<b>CHINA</b>					
Chengdu	1978			Spices, sausage, garlic	
Shanghai	1986			Apples, Potatoes, Onions, Garlic	
Zhengzhou	1986			Tomatoes	
Nanjing	1987				
Jinan	1987				
Lanzhou	1988				
Beijing	1988				
Tienjin	1988				
Daqing	1988				
Jianou	1991			Rice, Garlic, Spices	
Beijing	1995				
Dalian	1998				
Zhongshan	1999				
Inner Mongolia	1999				
Shuanglin	2000				

### Installation of Electron Accelerators for Industrial Purpose

No.	Application	Location	Machine Ratings	Years
<b>China</b>				
1	Heat shrinkable	Jilin Radiation	3.0MV 40mA	1984
2	Heat shrinkable	Engineering Physics	3.0MV 10mA	1987
3	Wire & cable	Tianshui Cable	2.2MV 25mA	1989
4	Wire & cable	Yantai Cable	2.0MV 20mA	1991
5	Wire & cable	Xian Wire	2.0MV 30mA	1993
6	Heat shrinkable/cable	Taiyuan	2.5MV 30mA	1993
7	Wire & cable	Sichuan Cable	2.0MV 10mA	1993
8	Heat shrinkable	Chengdu Shuangliu	2.0MV 10mA	1993
9	Wire & cable	Changshou Cable	2.5MV 20mA	1994
10	Wire & cable	Liyang Cable	2.5MV 20mA	1994
11	Heat shrinkable	Changchun Chemistry	2.5MV 30mA	1994
12	Wire & cable	Xinhua Cable	1.5MV 40mA	1995
13	Wire & cable	Xinhua Cable	1.5MV 30mA	1995
14	Wire & cable	Guangdong cable	2.5MV 40mA	1995
15	Heat shrinkable	Nuclear Technology	2.0MV 20mA	1995
16	Wire & cable	Kunming Cable	2.5MV 30mA	1995
17	Wire & cable	Shanghai Cable	2.5MV 33mA	1995
18	Wire & cable	Huangshi Cable	2.5MV 40mA	1995
19	Wire & cable	Shenyang Cable	2.0MV 10mA	1995
20	Heat shrinkable	Dayu Shrink-tube	2.0MV 10mA	1995
21	Heat shrinkable	Dayu Shrink-tube	2.0MV 10mA	1995
22	Heat shrinkable	Tianjin Tech-Physics	2.0MV 10mA	1995
23	Wire & cable	Yangzhong Cable	2.0MV 10mA	1995
24	Wire & cable	Jiangxi Cable	2.0MV 10mA	1995
25	Wire & cable	Shanghai-minhang Cable	2.5MV 30mA	1996
26	Heat shrinkable	Changchun Chemistry	1.5MV 40mA	1997
27	Heat shrinkable	Changchun Chemistry	1.5MV 40mA	1997
28	Heat shrinkable	Changchun Chemistry	1.5MV 40mA	1997
29	Heat shrinkable	Changchun Chemistry	1.5MV 40mA	1997
30	Wire & cable	Tianjin Cable	2.5MV 20mA	1997
31	Wire & cable	Lanxi Cable	2.5MV 40mA	1997
32	Wire & cable	Huaian Cable	2.5MV 25mA	1997
33	Flue gas	Chengdu Power	800kV 400mAX2	1997
34	Wire & cable	Zhengzhou Cable	2.5MV 30mA	1998
35	Wire & cable	Zhunhua Cable	2.5MV 25mA	1999
36	Heat shrinkable	Chengdu Shuangliu	3.0MV 30mA	2000
37	Heat shrinkable	Shenzhen Plastic	2.5MV 30mA	2000
38	Wire & cable	Sijiazhuang Cable	2.5MV 20mA	2001
<b>Indonesia</b>				
1	R&D(Curing)	PATIR	*1 300kV 50mA	1984
2	R&D(Cross-linking)	PATIR	*1 2.0MV 10mA	1993
3	Tire	GT	*2 500kV 150mA	1998

\*1: Center for the Application of Isotopes and Radiation Technology

\*2:PT Gajah Tunggal

No.	Application	Location	Machine Ratings	Years
<b>Korea</b>				
1	Wire & cable	LG Cable	750kV 65mA	1984
2	Wire & cable	LG Cable	1.5MV 65mA	1987
3	Wire & cable	LG Cable	1.0MV 100mA	1988
4	Wire & cable	LG Cable	2.0MV 50mA?	2000
5	Wire & cable	LG Cable	1.0MV 100mA?	2000
6	Wire & cable	Taihan Electric Wire	1.5MV 65mA	1988
7	Wire & cable	Dongyang Cable	1.0MV 50mA	1996
8	Heat shrinkable	Daewon Cable	1.0MV	1991
9	Tube	Daeryak Industry	1.0MV	1998
10	Wire & cable	Hankok KDK	1.0MV?	1997
11	Wire & cable	KyungShin Co.	1.0MV 65mA	1990
12	Tire	Hankok Tire	500kV 150mA	1993
13	Tire	Hankok Tire	500kV 150mAX2sets	1996
14	Tire	Kumho & Co.	800kV 100mAX2sets	1990
15	Foamed polymer	Youngbo Chemical	500kV 100mA	1990
16	Foamed polymer	Youngbo Chemical	1.0MV 100mA	1998
17	Foamed polymer	Tongil Ind.	800kV 65mA	1992
18	Curing	Tetrapack	175kV 300mA	
19	Cross-linking	Ceratech Co.	1.0MV 50mA?	
20	Waste water	Dyeing Complex	1.0MV 40mA?	1998
21	R & D	KAERI	300kV 25mA	1975
22	R & D	KAERI	2.0MV 45mA	2000
23	R & D/Service	EB Tech	1.0MV 40mA	
24	R & D/Service	EB Tech	1.0MV 40mA	
25	R & D	Youngnamu Univ.	800kV 35mA	1998
<b>Malaysia</b>				
1	R&D(Curing)	MINT	*1 200kV 20mA	1991
2	R&D(Cross-linking)	MINT	*1 3.0MV 30mA	1991
3	Wire & cable	Sumitomo	*2 800kV 100mA	1995
4	Wire & cable	Sumitomo	*2 2.0MV 50mA	2001
5	Packaging film	W.R.Grace	550kV 60mAX2	1996
6	Packaging film	S.K.Ploymer	150kV 460mA	1997
*1:Malaysian Institute for Nuclear Technology Research (MINT)				
*2:Sumitomo Electric Interconnect products, Johor				
<b>The Philippines</b>				
1	Sterilization	Terumo	10.0MV 28kW	2000
2	Tire	Yokohama	500kV 100mA	1998
<b>Thailand</b>				
1	Sterilization	Thai Klinipro	2.4MV 10kW	1997
2	Gem stones	IBA S&I	15MV 8.5kW	2000
3	Foamed polymer	Sekisui Thai	800kV 100mA	1997
<b>Vietnam</b>				
No installation of electron accelerator				

### Installation of Electron Accelerators in Japan

Application	Low Energy $E \leq 300\text{keV}$	Medium Energy $300\text{keV} < E \leq 3\text{MeV}$	High Energy $3\text{MeV} < E \leq 10\text{MeV}$	Total
Wire & cable	1	51	0	52
Foamed polymer	4	12	0	16
Heat shrinkable	15	17	1	33
Tire	3	20	0	23
Radiation curing	46	2	0	48
Flue gas & waste water	1	7	0	8
Sterilization	3	2	6	11
Irradiation service	7	11	4	22
Research and development	120	2	1	123
<b>Total</b>	<b>200</b>	<b>124</b>	<b>12</b>	<b>336</b>