Disinfection of *Bacillus cereus* in different food matrix using gamma irradiation

A Dissertation Submitted to East West University in Partial Fulfillment of the Requirements for the degree of Bachelor of Science in Genetic Engineering and Biotechnology

Submitted By

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CERTIFICATE

This is to certify that thesis entitled **"Disinfection of *Bacillus cereus* in different food matrix using gamma irradiation"** by **Md. Shohanur Rahman,** ID.: 2016-1-77-046, to be submitted to the Department of Genetic Engineering and Biotechnology, East West University, Aftabnagar, Dhaka-1212, in partial fulfillment of the requirement for the Degree of Bachelor of Science. This work was carried out under our supervision and the content of the thesis have been approved and recommended for the award of Bachelor degree.

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List of Abbreviations

^{o}C	Degree Celsius
^{60}Co	Cobalt-60
AERE	Atomic Energy Research Establishment
App.	Approximate
Cfu/gm or cfu/g or CFU g^{-1}	Colony forming unit/gram
et al	*et alliori* (and other)
e.g.	example gratita (for example)
i.e.	id *est* (that is)
g or gm	Gram
IFRB	Institute of Food and Radiation Biology
IAEA	International Atomic Energy Agency
kGy	Killo Gray
OD	Optical density
%	Percentage
pH	Negative logarithm of hydrogen

Abstracts

Bacillus cereus is gram positive facultative anaerobic bacterium. It is a foodborne pathogen responsible for human food poisoning. In this study, growth and disinfection of *B. cereus* KTCC-11204 and *B. cereus* KTCC-400935 were examined in different food matrix using gamma radiation. Both of these strains were inoculated separately into fried rice and pasta samples which were collected from Dhaka. After appropriate incubation fried rice and pasta were irradiated using gamma radiation at 1 kGy, 3 kGy, 5 kGy, 7 kGy, 8 kGy and 9 kGy. Results showed that when the radiation doses were increased in fried rice, the number of colonies of *B. cereus* were significantly decreased. Both of the strains in fried rice were completely eliminated at 8 kGy of gamma radiation dose. Meanwhile, pasta samples showed similar results and complete elimination was obtained at 8 kGy gamma radiation dose. In both fried rice and pasta showed higher growth of KTCC-11204 than KTCC-400935. This study indicates that *B. cereus* can grow in prepared foods that can be potentially disinfected using gamma radiation which might be helpful in improving food safety of prepared foods.

1. Introduction

1.1: Background:

Food poisoning, also called foodborne illness, is illness caused by eating contaminated food. Infectious organisms — including bacteria, viruses and parasites — or their toxins are the most common causes of food poisoning. Contamination of food can happen at any point of production: growing, harvesting, processing, storing, shipping or preparing. Cross-contamination — the transfer of harmful organisms from one surface to another — is often the cause.

B. cereus is a toxin-producing bacteria that is one of the most common causes of food poisoning, also called "fried rice syndrome." It can cause two types of food poisoning known as the emetic and the diarrheal types. In a small number of cases both types of symptoms are recorded, probably due to production of both types of toxins.

There has been some debate about whether or not the enterotoxin(s) can be performed in foods, and cause an intoxication. The enterotoxin(s) can be performed, the number of *B. cereus* cells in the food would be at least two orders of magnitude higher than that necessary for causing food poisoning, and such products would no longer be acceptable to the consumer. The emetic type is caused by a heat-stable toxin, named cereulide, preformed in the food.

The dominating type of disease caused by *B. cereus* differs from country to country. In Japan the emetic type is reported about 10 times more frequently than the diarrhoeal type, while in Europe and North America the diarrhoeal type is the most frequently reported. Since *B. cereus* food poisoning is not a reportable disease in any country, there are very few figures given of the total number of these kinds of food poisoning.

B. cereus causes self-limiting (24–48 h) food-poisoning syndromes. Besides food related illnesses *B. cereus* may also cause non-gastrointestinal disease like endocarditis and endophthalmitis. The accurate number of food poisonings caused by *B. cereus* in different countries is not known because it is not a reportable illness and is not always diagnosed [1].

1.2: General Description of *Bacillus cereus*:

B. cereus is a Gram-positive, rod-shaped, facultatively anaerobic, motile, beta-hemolytic, spore forming bacterium commonly found in soil and food. *B. cereus* is a large, 1 x 3-4 µm. The specific name, *cereus*, meaning "waxy" in Latin, refers to the appearance of colonies grown on blood agar. Some strains are harmful to humans and cause foodborne illness, while other strains can be beneficial as probiotics for animals. It was first successfully isolated in 1969 from a case of fatal pneumonia in a male patient and was cultured from the blood and pleural fluid [2]. 16s rRNA comparison reveals *B. cereus* to be most related to *Bacillus anthracis*, the cause of anthrax, and *Bacillus thuringiensis*, an insect pathogen used as pesticide. Although they have similar characteristics, they are distinguishable as *B. cereus* is most motile, *B. thuringiensis* produces crystal toxins, and *B. anthracis* is nonhemolytic [3].

B. cereus is mesophilic, growing optimally at temperatures between 20°C and 40°C, and is capable of adapting to a wide range of environmental conditions. It is distributed widely in nature and is commonly found in the soil as a saprophytic organism [4]. *B. cereus* is also a contributor to the microflora of insects, deriving nutrients from its host, and is found in the rhizosphere of some plants.

B. cereus is an opportunistic human pathogen and is occasionally associated with infections, causing periodontal diseases and other more serious infections.

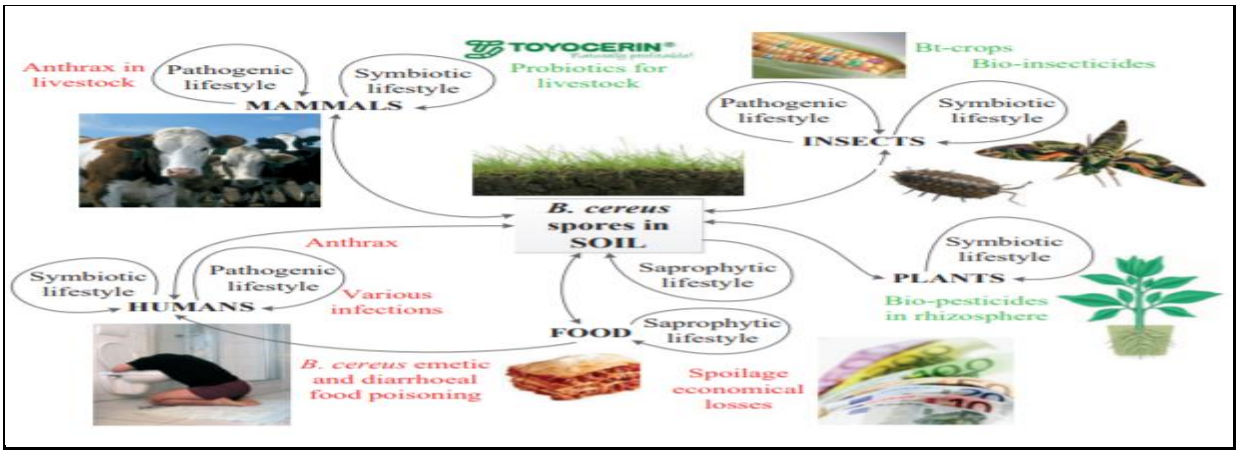

Figure 1: *B. cereus* group display a wide variety of different lifestyles and life cycles.

In different hosts, leading to a number of divergent applications and diseases, depending on the environmental context, the hosts and the specific characteristics of the strains.

1.3: Taxonomy:

Taxonomically the genus *Bacillus* is difficult because of the heterogeneity in the physiologic characteristics of the species. Differentiation between *B. cereus* and related species is complicated by similarities in phenotypic and genetic properties [56]. Greater than 99% sequence similarity in the primary structures of 16S rRNA has been reported among *Bacillus anthracis*, *B. cereus*, *B. mycoides*, and *B. thuringiensis* [74]. There are some distinguishing characteristics in these closely related species. *B. anthracis* has two virulence plasmids, *B. thuringiensis* produces crystalline parasporal inclusions and delta-endotoxin, and the colonies of *B. cereus* var. *mycoides* show rhizoid growth on blood agar [56]. Based on very high similarity all these species have been regarded to be variations of *B. cereus*.

1.4: Classification:

Domain: **Bacteria**

Kingdom: **Eubacteria**

Phylum: **Firmicut**

Class: **Bacilli**

Order: **Bacillales**

Family: **Bacillacae**

Genus: **Bacillus**

Species: ***Bacillus cereus***

1.5: Cell Structure:

B. cereus is a 1 x 3-4 µm, rod shaped, Gram- positive bacterium. Its cell structure consists of an inner membrane and a thick peptidoglycan which functions to maintain cell shape. The polysaccharide portion makes up 50% percent of the cell wall and consists of a neutral polysaccharide composed of N-acetylglucosamine, N-acetylmannosamine (ManNac), N-acetylgalactosamine and glucose in a molar ratio of 4: 1: 1: 1 [5]. The acidic portion of the cell wall is characteristic in having a repeating tetrasaccharide unit [5]. 5% of the cell wall is made up of techoic acids consisting of N-acetylglucosamine, galactose, glycerol, and phosphorus in a molar ratio of 1: 1.4: 1: 1 [5]. The linkage between the polysaccharide and peptidoglycan is a muramic acid 6-phosphate. The peptidoglycan of some *B. cereus* strains are unique with only a few oligomers present, the cross-linked muropeptides are dimmers, and many of the muropeptide lack the N-acetyl group [6]. These distinguishing features affect cell surface charge which contributes to the attachment of an outer capsule or an S-layer in pathogenic strains.

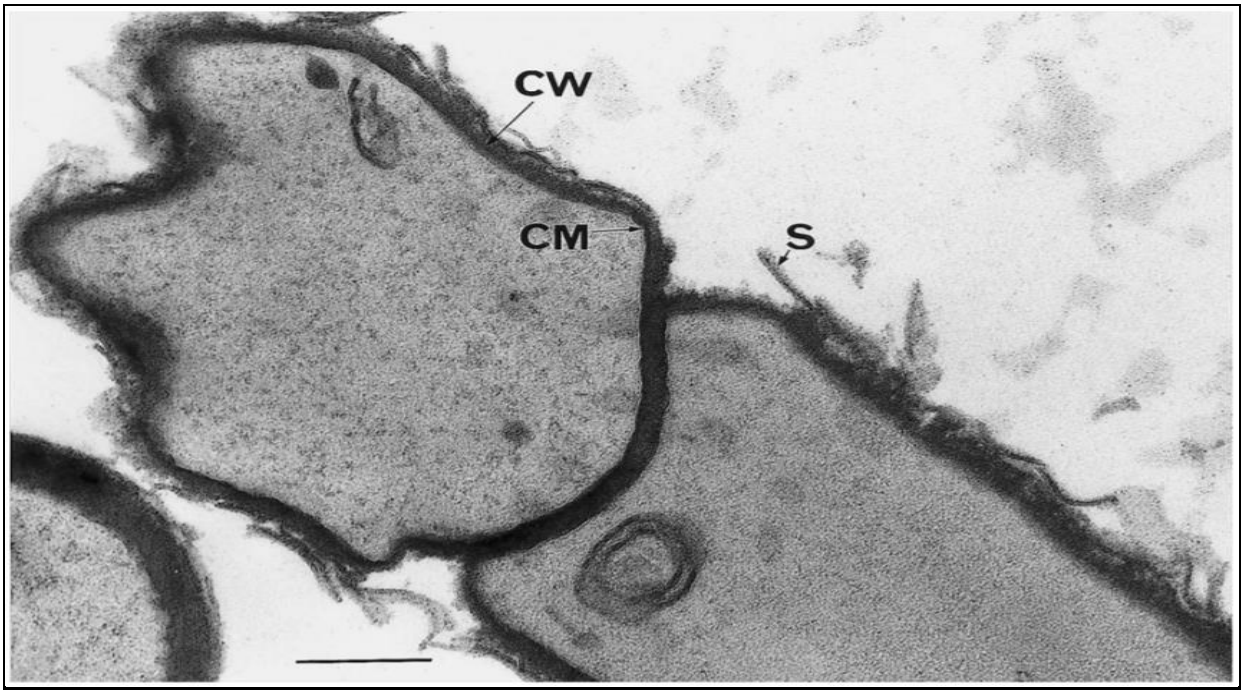

Figure 2: Ultrastructure of a *B. cereus* sporangium. Figure shows; CM = cytoplasmic membrane, CW = cell wall, S = surface layer. Bar in this and subsequent micrographs represents 0.2 µm.

B. cereus have also a glycoprotein S-layer over its peptidoglycan which consists of proteinaceous paracrystalline arrays and covers the cell surface. The S-layer is involved in the virulence of *B. cereus* and functions to promote interactions with human polymorphonuclear leucocytes [7]. This proteinaceous layer enhances its resistance to radiation.

B. cereus is motile by means of flagella and exhibits two types of motility including swimming and swarming, depending on the environment. Single cells exhibit swimming motility by means of short flagellated rods and on the other hand, swarming is a collective movement of swarm cells with flagellum that is observed to be three to four times longer, and also forty times more flagellated than single swimming cells [8].

1.6: Metabolism:

B. cereus is a facultative aerobe so it can utilize oxygen as a terminal electron accepter, but also has methods of anaerobic respiration as a mechanism of energy release. Whole genome sequencing revealed genes encoding for metabolic enzymes such as NADH dehydrogenases, succinate dehydrogenase, complex III, non-proton-pumping cytochrome bd quinol oxidases, and proton-pumping oxidases such as cytochrome c oxidase and cytochrome aa3 quinol oxidase [9].

In aerobic respiration, reducing equivalents produced from glycolysis and the Krebs cycle are reoxidized by the electron transport chain, creating a proton motive force and ATP by ATP synthase [9]. In anaerobic respiration, *B. cereus* utilizes fermentation to generate energy. Fermentation recycles NAD+ by reducing pyruvate and produces lactate and ethanol [9]. ATP is generated by substrate level phosphorylation.

B. cereus can metabolize a variety of compounds including carbohydrates, proteins, peptides and amino acids for growth and energy. Some of the major products produced from carbon sources such as sucrose or glucose during anaerobic respiration include L-lactate, acetate, formate, succinate, ethanol, and carbon dioxide [10]. During nitrate respiration, nitrate reductase converts nitrate into nitrite which is converted to ammonium by nitrite reductase [10].

1.7: Reproduction:

B. cereus undergoes reproduction by the means of asexual reproduction, more specifically binary fission. Binary fission is the asexual reproduction method used by all prokaryotes, it occurs when a single parent cell undergoes mitosis and produces two equally sized daughter cells. Both daughter cells produced have the potential to grow to the size of the parent cell.

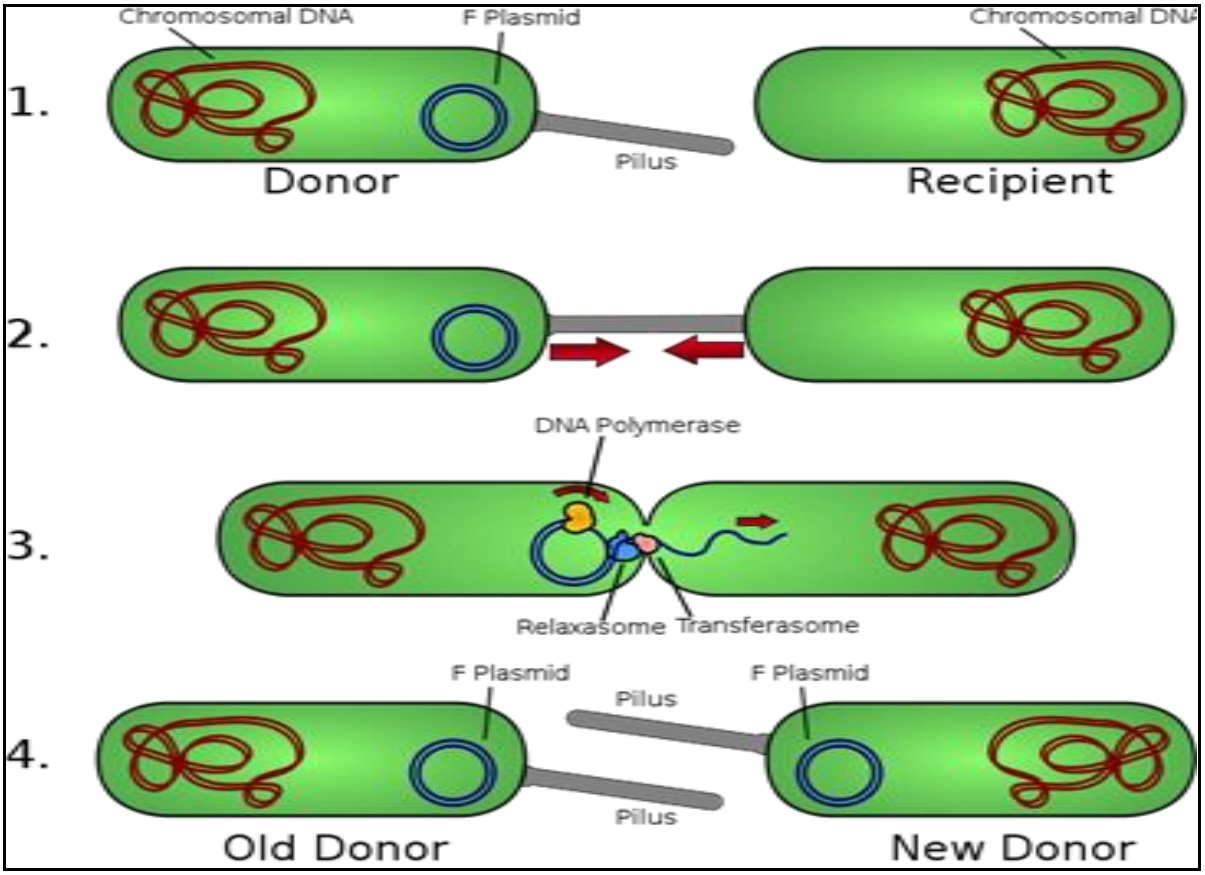

Figure 3: General reproduction mechanism of *B. Cereus*.

Conditions for which Bacillus cereus reproduces are not very specific. It can produce in the sediment, food, or an infected host such as us. Though optimal conditions for growth are as follows: temperature between twenty-eight and thirty-five degrees Celsius, pH range from 4.3 to 9.3, and oxygen must be present.

At 30 °C (86 °F), a population of *B. cereus* can double in as little as 20 minutes or as long as 3 hours, depending on the food product [11].

Table 1: Population doubling time of *B. cereus* at different food matrix

Food	Minutes to double, 30 °C (86 °F)	Hours to multiply by 1,000,000
Milk	20–36	6.6 - 12
Cooked rice	26–31	8.6 - 10.3
Infant formula	56	18.6

1.8: Genome:

B. cereus has a circular chromosome measuring 5,411,809 nt in length and was completely sequenced using the shotgun sequencing method [12]. The genome structure of *B. cereus* consists of 5481 genes, 5234 protein coding, 147 structural RNAs, and 5, 366 RNA operons [13]. An interesting gene cluster found within its genome encodes for arginine deiminase metabolic pathway. This cluster is predicted to have a role in its survival, enabling it to be resistant to acidic conditions in a similar manner as *Streptococcus pyogenes* [14]. Additionally, *B. cereus* has a nine gene urease gene cluster that encodes for proteins, blasticidin S deaminase, and an S-layer protein [14]. The urease enzyme increases its vigor in acidic conditions and is similar to the urease found in other bacteria that is required for colonization of the human stomach [14].

Genes present within the chromosome associated with *B. cereus* virulence include genes encoding for non-hemolytic enterotoxins, channel-forming type III hemolysins, phospholipase C, a perfringolysin O (listeriolysin O), and extracellular proteases [15]. The *hbl* operon, an RNA transcript of 5.5 kb, transcribes all three proteins of the hemolysin BL enterotoxins associated with food poisoning. These genes along with other genes encoding for metabolic enzymes, proteins involved in motility and chemotaxis, proteins involved in sporulation, and cellular transporters are all regulated by the *plcR* gene [16]. The *plcR* gene is also required for full virulence of *B. cereus*, and is often the target of antimicrobial drugs. Another gene found on its chromosome is the *gerA*gene which is essential for sporulation when nutrients are depleted, and is responsible for spore germination stimulated by L-alanine and ribosides [9]. It also has 18-23 genes that encode for peptide and amino acid ABC transporter-ATP binding proteins which suggests that amino acids, proteins, and peptids are preferred nutrient sources [16].

1.9: Food irradiation:

It is the process of exposing food and food packaging to ionizing radiation. Ionizing radiation, such as from gamma rays, x-rays, or electron beams, is energy that can be transmitted without direct contact to the source of the energy (radiation) capable of freeing electrons from their atomic bonds (ionization) in the targeted food. The radiation can be emitted by a radioactive substance or generated electrically. This treatment is used to improve food safety by extending product shelf-life (preservation), reducing the risk of foodborne illness, delaying or eliminating sprouting or ripening, by sterilization of foods, and as a means of controlling insects and invasive pests. Food irradiation is a technology that improves the safety and extends the shelf life of foods by reducing or eliminating microorganisms and insects.

Although consumer perception of foods treated with irradiation is more negative than those processed by other means, because people imagine that the food is radioactive or mutated, these thoughts don't agree with the understood mechanism by which irradiation works. But all independent research, the U.S. Food and Drug Administration (FDA), the World Health

Organization (WHO), the Centers for Disease Control and Prevention (CDC), and U.S. Department of Agriculture (USDA) have performed studies that confirm irradiation to be safe [17].

Food irradiation is permitted by over 60 countries, with about 500,000 metric tons of food annually processed worldwide. In Austria, Germany, and many other countries of the European Union only dried herbs, spices, and seasonings can be processed with irradiation and only at a specific dose, while in Brazil all foods are allowed at any dose [18].

Figure 4: Radura Sign. The international Radura logo, used to show a food has been treated with ionizing radiation.

1.10: Sources of Irradiation:

Irradiation is the process by which an object is exposed to radiation. The exposure can originate from various sources, including natural sources. **There are three sources of radiation approved for use on foods.**

(1) Gamma Irradiation:

Gamma irradiation is produced from the radioisotopes **cobalt-60** and **caesium-137**, which are derived by neutron bombardment of cobalt-59 and as a nuclear source by-product, respectively. **Cobalt-60** is the most common source of gamma rays for food irradiation in commercial scale facilities as it is water insoluble and hence has little risk of environmental contamination by leakage into the water systems. As for transportation of the radiation source, cobalt-60 is

transported in special trucks that prevent release of radiation and meet standards mentioned in the Regulations for Safe Transport of Radioactive Materials of the International Atomic Energy Act [19]. **Caesium-137** is water soluble and poses a risk of environmental contamination. Insufficient quantities are available for large scale commercial use. An incident where water-soluble **caesium-137** leaked into the source storage pool requiring NRC intervention has led to near elimination of this radioisotope. **Gamma** irradiation is widely used due to its high penetration depth and dose uniformity, allowing for large-scale applications with high through puts. Gamma radiation is used routinely to sterilize medical, dental, and household products and is also used for the radiation treatment of cancer.

(2) X-rays:

X-rays are produced by bombardment of dense target material with high energy accelerated electrons giving rise to a continuous energy spectrum [19]. This process is also known as **bremsstrahlung-conversion**. Heavy metals, such as **tantalum** and **tungsten**, are used because of their high atomic numbers and high melting temperatures. Tantalum is usually preferred versus tungsten for industrial, large-area, high-power targets because it is more workable than tungsten and has a higher threshold energy for induced reactions. X-rays have high penetration depths and high dose uniformity but they are a very expensive source of irradiation as only 8% of the incident energy is converted into X-rays.

(3) Electron beam:

Treatment of electron beams is created as a result of high energy electrons in an accelerator that generates electrons accelerated to 99% the speed of light [19]. This system uses electrical energy and can be powered on and off. The high power correlates with a higher throughput and lower unit cost, but electron beams have low dose uniformity and a penetration depth of centimeters. Therefore, electron beam treatment works for products that have low thickness. Electron beam (or e-beam) is similar to X-rays and is a stream of high-energy electrons propelled from an electron accelerator into food.

1.11: Benefits of Food Irradiation:

Irradiation can be effect direct, caused by reactive oxygen-centred (OH) radicals originating from the radiolysis of water or indirect on organisms and food products. An indirect effect (the damage to the nucleic acids) occurs when radiation ionizes a neighboring molecule, which in turn reacts with the genetic material. The optimum dose is a balance between that what is needed and that what can be tolerated by the products [20].

1. Microorganisms, insect gametes, and plant meristems are prevented from their reproduction, which consequently results in various preservative effects as a function of the absorbed radiation dose [21].

2. The size of the DNA molecule generally increases with the complexity of an organism, viruses are more radiation resistant than other organisms [22]. The combination of irradiation with heating can be used successfully to inactivation of viruses [22].

3. Insects, mites and other such pests are higher level, multicellular organisms responsible for considerable loss of fresh produce and grains. Also, they can provide as vectors for carrying pathogenic bacteria and parasites [23].The best control of insects in agricultural products can be achieved by using fumigants. Radiation has been suggested as an alternative to fumigants.

4. Low and medium doses induce only a small breakdown of food proteins into lower molecular weight protein parts and amino acids. As a result, experiments indicated that such treatments cause less chemical reactions than steam heat sterilization [24].

5. Modification of physical properties by reduces rehydration time of dehydrated vegetables.

6. Irradiation retard the natural decay of fruits and vegetables, thus extending shelf life.

7. Pathogens such as Salmonellae which causes food poisoning can be eliminated from egg, dairy products, poultry and meat.

1.12: Objectives:

1. Examination of the effect gamma radiation on *Bacillus cereus* in different food matrix.

2. Comparison of the growth and disinfection of gamma radiation in two different *B. cereus* strains as well as food matrix.

2. Literature Review

2.1: Emetic and diarrhoeal food poisoning caused by *B. cereus*:

B. cereus is becoming one of the most important causes of food poisoning in the industrialized world. *B. cereus* causes two different types of food poisoning: the diarrhoeal type and the emetic type. The diarrhoeal type of food poisoning is caused by complex enterotoxins [25], produced during vegetative growth of *B. cereus* in the small intestine [26], while the emetic toxin is produced by growing cells in the food [27]. *B. cereus* is not a competitive microorganism, but grows well after cooking and cooling (<48°C). The heat treatment will cause spore germination, and in the absence of competing flora, *B. cereus* grows well. In a small number of cases both types of symptoms are recorded, probably due to production of both types of toxins. There has been some debate about whether or not the enterotoxin(s) can be preformed in foods, and cause an intoxication. Reviewing the literature it is obvious that the incubation time is a little too long for that (>6 h; average 12 h) [27], and in model experiments it has been shown that the enterotoxin(s) is degraded on its way to the ileum [26].

Although the enterotoxin(s) can be preformed, the number of *B. cereus* cells in the food would be at least two orders of magnitude higher than that necessary for causing food poisoning [28], and such products would no longer be acceptable to the consumer.

The characteristics of the two types of *B. cereus* food poisoning are given in below: [27, 29]

Table 2: Characteristics of two types of toxic syndrome caused by *B. cereus*.

Subjects	Diarrhoeal syndrome	Emetic syndrome
Infective dose	10^5–10^7 (total)	10^5–10^8 (cells g^{-1})
Toxin produced	In the small intestine of the host	Preformed in foods
Type of toxin	Protein	Cyclic peptide
Incubation period	8–16 h (occasionally >24 h	0.5–5 h
Duration of illness	12–24 h (occasionally several days)	6–24 h
Symptoms	Abdominal pain, watery diarrhoea and occasionally nausea	Nausea, vomiting and malaise (sometimes followed by diarrhoea, due to additional enterotoxin production).
Foods most frequently implicated	Meat products, soups, vegetables, puddings/sauces and milk/milk products	Fried and cooked rice, pasta, pastry and noodles

According to the European Food Safety Authority (EFSA) reports on food-borne outbreaks in the EU, *B. cereus* was responsible for only 1.88% of the reported food-borne outbreaks in 2010 (EFSA, 2012) [30]. However, *B. cereus* was identified as the causative agent in 56% of the food-

borne outbreaks reported in 2011 to the Laboratoire Central des Services veterinaires (situated at Anses, Maisons-Alfort) in France for which the causative agent was identified, which was the case for 60 of the 197 outbreaks in total (Anses, Laboratoire de securite des aliments de Maisons-Alfort, pers. commun.). *B. cereus* is underestimated as a food-borne pathogen due to a number of causes. Firstly, underreporting exists due to the mild and transient symptoms of the illness. Secondly, *B. cereus* food poisoning is not classified by EFSA as a zoonosis, a disease transmitted through animals, and therefore has received less attention in reporting and surveillance compared with zoonotic agents. Thirdly, atypical and emetic strains, which often display no or low lecithinase and haemolytic activity, are not detected with the standard enumeration of *B. cereus* by plating on MYP according to ISO7932:2004 [31, 32]Fourthly, food products implicated in food poisoning outbreaks are likely to contain multiple *B. cereus* strains [33]. To identify the strain responsible for the illness, multiple *B. cereus* colonies of different morphology should be selected for further investigation and, ideally, linked to identical isolates from the stool and/or vomit of patients.

Few studies regarding the prevalence of *B. cereus* in food distinguished between the cellular and spore forms. Therefore, it is difficult to estimate the relative prevalence of *B. cereus* vegetative cells and spores in food products at the time of consumption. *B. cereus* spores are definitely expected to be present in food, but their prevalence may vary among different food types. For example, 37% of the *B. cereus* present in Belgian retail lasagne were spores, in comparison with only 6% in raw rice [34]. Similarly, retail raw rice from the United States often (53%) contained *B. cereus* spores, albeit in very low numbers (33 spores/g on average) [35].

It is overall assumed that only consumption of food containing between 10^5 and 10^8 *B. cereus* cells and spores will cause disease [36, 37]. Emetic food poisoning is usually caused by preformed cereulide in food, because this emetic toxin is not inactivated during food processing or gastrointestinal passage due to its high resistance against heat treatments, extreme pH values and protease activities [38-40]. As a consequence, ingestion of living *B. cereus* is not necessarily required to experience this type of illness. In contrast, diarrhoeal food poisoning is not caused by preformed enterotoxins in food, but by viable vegetative *B. cereus* cells producing enterotoxins in the small intestine, because spores produce no enterotoxins, and these proteins are rapidly (0.5 h)

degraded under gastrointestinal conditions by proteases present in the host's digestive secretions, even at their basic fasted levels [41-44]. The current hypothesized course of *B. cereus* diarrhoeal food poisoning is presented in Figure -5.

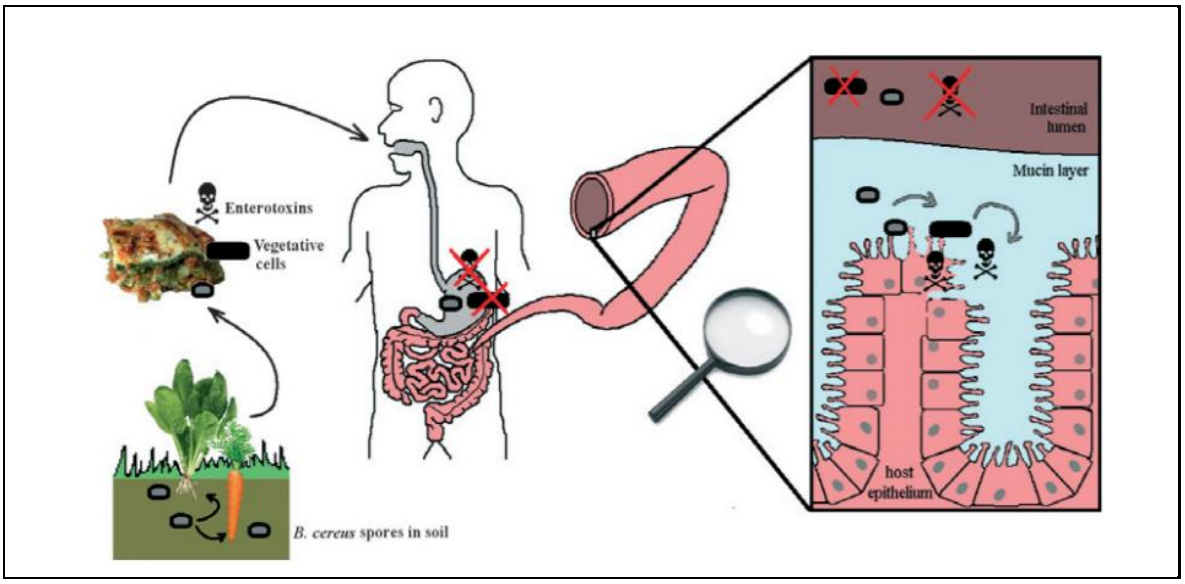

Figure 5: General mechanism of *B. cereus* inside human body.

2.2: *B. cereus* foodborne outbreaks:

B. cereus is a gram-positive or gram-variable, rod-shaped, aerobic-to-facultative, spore-forming bacterium. Its bacterial spores do not swell the sporangium and sporulate only under aerobic conditions [45]. *B. cereus* is generally considered a mesophilic microorganism, with a temperature range for growth between 10 and 50 °C (with an optimum between 28 and 37 °C). Furthermore, only a few strains can multiply below 7 °C and above 45 °C. The pathogen can grow at pH values from 4.3 to 9.3 (with an optimum between 6.0 and 7.0) and at a minimum of water activity (aw) of 0.92. *B. cereus* spores are moderately heat-resistant and survive freezing and drying. *B. cereus* is ubiquitous in nature, and living cells can be found in soil (where toxins can persist), water, vegetables, decaying matter, the intestinal tract of animals, and insects [45-47].

The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks reported a total of 287 outbreaks caused by *B. cereus* toxins involving 3073 cases (about 8% hospitalization) in European Member States (MSs) in 2014 [48], whereas 291 outbreaks involving 3131 cases (with 3% hospitalization) were reported by nine MSs in 2015. In the year 2000, 173 people were intoxicated by *B. cereus* toxins after they attended two banquets in Pizza (Italy). All of the infected people had nausea and watery diarrhea. Ghelardi et al. (2002) conducted a microbiological investigation that involved food, environmental samples and stool collected from the patients (23) who were hospitalized. Identification and characterization of the toxigenic *B. cereus* isolated from the samples was also carried out. Food samples (cakes) showed microbial loads reaching 10^2 colony forming units (cfu)/gm [49]. In 2006, two different outbreaks involving 17 children and 1 child, respectively, occurred in a day care center in Germany [50]. The first outbreak was caused by the consumption of a rice dish with vegetables that had a load of 10^4cfu/g of *B. cereus*. The gastrointestinal illness (vomiting) started about 75min after consumption of a small amount of the reheated food. All of the analyzed foods revealed the presence of the *B. cereus* emetic toxin (cereulide).

The diarrheal type of food poisonings have been reported in Hungary, Finland, Bulgaria, and Norway more often than the emetic type, which was prevalent in Japan and in the United Kingdom between 1950–1985 [27]. The percentages of foodborne illnesses caused by *B. cereus* differed from country to country. Between 1973–1985, *B. cereus* caused 17.8% of the total bacterial food poisonings in Finland, 11.5% in the Netherlands, 0.8% in Scotland, 0.7% in England and Wales, 2.2% in Canada, 0.7% in Japan, and 15.0% (between 1960–1968) in Hungary [27]. In Norway *B. cereus* was the most common microbe isolated from foodborne illnesses in 1990 [52]. The Taiwan Department of Health reported 74 outbreaks of foodborne illnesses caused by bacteria in 1994. In 14.9% of these outbreaks the causative pathogen was *B. cereus* [53]. In the United States *B. cereus* is not a common cause of foodborne disease, the incidence being only 1.3% of the bacterial food poisoning cases reported between 1972–1982 [27]. Nevertheless, some large outbreaks have occurred; for example, in 1989 over 100 people were affected [27, 51]. The number of reported cases in different countries is not comparable because of diversity in reporting procedures.

2.3: Risk food products:

The food product strongly influences the production of both emetic and diarrhoeal toxins through its specific nutritional composition, other intrinsic food properties and storage [54]. Emetic food poisoning usually involves food products with high starch contents, such as pasta, rice, mashed potatoes, bread and pastries, which stimulate the production and accumulation of the stable toxin cereulide [55, 56], although the majority (> 90%) of emetic *B. cereus* strains are unable to hydrolyze starch [57]. It is also important to note that under anaerobic conditions with < 1 to 2% O_2, cereulide production no longer occurs [58, 59]. Emetic *B. cereus* strains in the exponential growth phase can produce 0.004– 0.130 µg of cereulide per 10^6 cells, independent of sporulation [58, 60]. This means that populations in the range of 10^5 to 10^8 can produce sufficient amounts of toxin to cause gastrointestinal illness, because the estimated required dose of cereulide is between 0.02 and 1.83 µg per kg body weight [54]. In contrast, diarrhoeal food poisoning cases occur with a wide variety of food commodities. The influence of food type is less pronounced in these cases, because preformed enterotoxins in food are rapidly degraded during gastrointestinal passage. The food matrix significantly influences the gastrointestinal survival of *B. cereus* vegetative cells by modifying the acid resistance and bile resistance at 3.0 g L^{-1} [61, 62], while this influence is no longer observed for continuously decreasing gastric pH values and higher bile concentrations of ≥ 5.0 g L^{-1} [63].

In Belgium, 70% of the retail lasagne in Belgium contained *B. cereus*, as well as 77% of the bolognese sauce samples, 81% of the bechamel sauce, 20% of the cooked pasta, 100% of the raw rice, 15% of the fresh minced beef, 40% of the carrots, 35% of the celery, 30% of the Chinese cabbage and 5% of the bell pepper [34]. Screening of the food ingredients showed that *B. cereus* only occurred in herbs and spices, except for two positive samples of raw meat ($10^{2.5}$ CFU g^{-1}) and potato flakes ($10^{3.5}$ CFU g^{-1}). Although the majority (72%) of herbs and spices were free of *B. cereus* (< 10^2 CFU g^{-1}) and the contamination was usually at tolerable levels (97% 10^4 CFU g^{-1}), high levels of *B. cereus* in herbs and spices were sporadically detected (3% > 10^5 CFU g^{-1}). However, none of the finished products contained *B. cereus* at > 10^3 CFU g^{-1} after production or after storage at the time of consumption. In the Netherlands, REPFEDs and a wide variety of other retail food products were also contaminated with *B. cereus*, but the majority (> 99%) of the contamination levels were situated below the tolerance level of 10^5 CFU g^{-1} at the time of

consumption [64]. In the USA, 18% of retail seafood samples contained *B. cereus*, although usually (62%) at low levels (< 10^2 CFU g^{-1}) [65]. Also in Korea, *B. cereus* was frequently isolated from glutinous rice (37%), Job's tears (27%), barley (21%), brown rice (18%) and soybean sprouts (71%), although again at low levels 10^2 CFU g^{-1} [66, 67]. The overall prevalence of *B. cereus* in Australian retail food samples was surprisingly low, being absent (< 10^2 CFU g^{-1}) in 98% of the cases and most frequently found in chilled raw chicken (6% containing on average 2.0×10^4 CFU g^{-1}), frozen cooked meat pies (5% with 1.6×10^2 CFU g^{-1}) and pizzas (5% with 2.5×10^3 CFU g^{-1}) [68].

In addition, the storage temperature of the final food product is the major factor influencing the number and type of *B. cereus* present at the end of shelf life. For example, storage of zucchini puree at 4 °C for 21 days did not reveal any *B. cereus* present (< 5.0×10^1 CFU g^{-1}), while storage at 10 °C for 21 days resulted in 4.0×10^4 CFU g^{-1} of mesophilic and psychrotolerant soil strains and storage at room temperature for 5 days revealed 2.5×10^6 CFU g^{-1} mesophilic strains from soil and milk proteins [69, 70]. In conclusion, *B. cereus* is a ubiquitous environmental and food contaminant, which is present in a wide variety of retail food products worldwide, although seldom in high (>10^5 CFU g^{-1}) concentrations.

3. Materials and Methods

3.1: Bacterial Strain Culture:

In this experiment two bacterial strains were used which are *B. cereus* KTCC-11204 and *B. cereus* KTCC-400935. These two strains were collected from Institute of Food and Radiation Biology (IFRB) division of Atomic Energy Research Establishment (AERE), Ganakbari, Savar, Dhaka, Bangladesh. Here KTCC is the abbreviation of Korean Type Culture Collections. *B. cereus* KTCC-11204 create small and round shape colonies and *B. cereus* KTCC-400935 create large round shape colonies. These strains were kept at 4^0 C in refrigerator because of this temperature arrested the bacterial growth and these strains were used over a long period of time. Before every new procedure of bacteria were freshly cultured in petri dish according to streak plate method from the procedure and this mechanism ensured contamination free method.

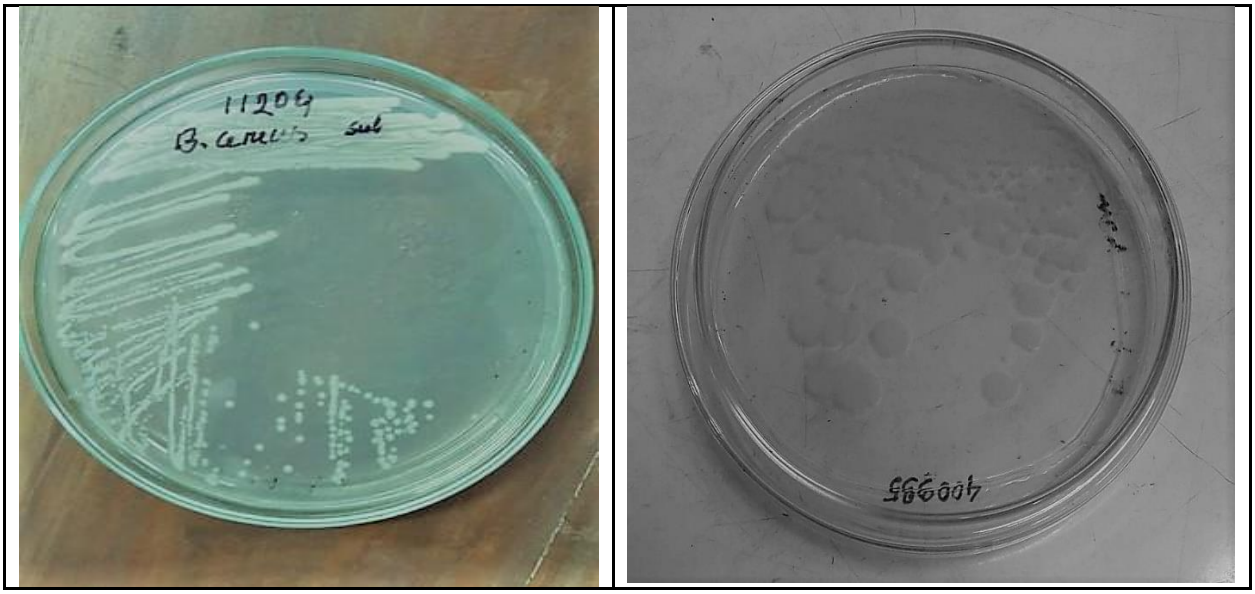

Figure 6: Streak plating of *B. cereus* KTCC-11204 and *B. cereus* KTCC-400935 in agar plate. Figure shows isolation of single colony that ensured contamination free mechanism for other methods.

3.2: Bacterial Growth:

For bacterial growth in this experiment used the nutrient broth. At first measured solid nutrient broth in a beaker for preparing broth media. Then distilled water was taken by using measuring cylinder and mixed with the solid nutrient broth. Took 10 ml liquid nutrient broth in a pipette and poured it into a test tube (repeat 6 times). Six test tubes each containing 10 ml of liquid broth media were sterilized by autoclaving at 121^0 C and 15 psi. After autoclave process, the sub-cultured bacillus cereus strain were mixing with the broth medium and vortexing properly. The test tubes were capped properly and stayed it in incubator for growing bacteria into test tubes about 24 hours at 30^0 C.

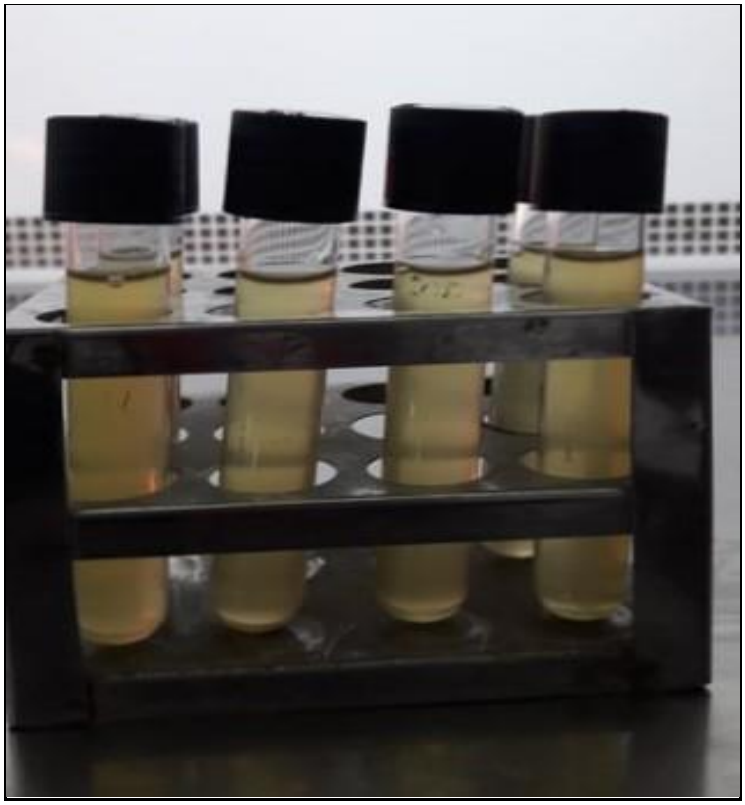

Figure 7: Subculture of *B. cereus* into liquid nutrient growth media.

3.3: Fried Rice:

Sample Collection and Preparation:

Fried rice was used in this experiment and it was collected from Savar, Dhaka, Bangladesh.

At first the fried rice was sterilized by gamma radiation at 25 kGy. The contamination free fried rice was measured at 10 grams in a bottle. This pathway followed and prepared more five bottles. Total six bottles contained 10 grams fried rice which were already contained 90 ml saline water each of bottles. Saline water was sterilized by fully automated autoclave machine at 121^0 C and 15 psi. . After that step, the bacterial culture (nutrient broth) mixed with the fried rice and saline water into the bottles properly.

Figure 8: Rice samples in saline water. Each bottle containing fried rice samples. These samples were prepared in laboratory and sterilized carefully. Each bottle contained 10 gm of fried rice and 90 ml of normal saline water.

3.4: Pasta:

Sample Collection and Preparation:

Pasta was used in this experiment and it was collected from Mirpur, Dhaka, Bangladesh.

At first the pasta was sterilized by gamma radiation at 25 kGy . The contamination free pasta was measured at 10 grams in a bottle. This pathway followed and prepared more five bottles. Total six bottles contained 60 grams pasta and that were already contained 90 ml saline water each of bottles. Saline water was sterilized by fully automated autoclave machine at 121^0 C and 15 psi. . After that step, 10 ml bacterial culture (nutrient broth) mixed with the pasta and saline water into the bottles properly.

Figure 9: Pasta sample. Each bottle containing pasta samples. These samples were prepared in laboratory and sterilized carefully. Each bottle contained 10 gm of pasta and 90 ml of normal saline water.

3.5: Incubation:

These procedure were carried out in the sterile condition. After measuring fried rice and pasta in the bottle, the bacteria which grown in nutrient broth were mixed with the fried rice and pasta. These whole process was carried out inside lamina air flow. Then the sample containing bottles were kept in incubator at 28^0 C for 24 hours.

3.6: Irradiation Treatment:

Irradiation of the samples was carried out by using Cobalt-60 Panoramic Research Irradiator of Institute of Food and Radiation Biology (IFRB), Atomic Energy Research Establishment (AERE), Bangladesh Atomic Energy Commission, Bangladesh. The activity of the irradiator was 38.53 kCi on the date of irradiation of these samples. Primarily, measurement of dose-rate was performed by Fricke dosimetry system. Fricke dosimeters were prepared following standard protocols; by dissolving 0.392 of ferrous ammonium sulphate ($Fe(NH_4)_2(SO_4)_2.6H2O$) and 0.058 g of sodium chloride (NaCl) in 12.5 mL of 0.4 mol/L sulphuric acid (H_2SO_4). The volume of the solution was made up to 1 L in a volumetric flask with 0.4 mol/L H_2SO_4 at 25°C. Therefore, the concentrations of final solution were 0.001 M ferrous ammonium sulphate, 0.001 M sodium chloride and 0.4 mol/L sulphuric acid. Freshly prepared Fricke solution was poured in screw-cap vial and placed in the radiation field with samples for certain amount of time. After completion of irradiation, absorbance of irradiated Fricke solution was measured using spectrophotometer at 303 nm; the unirradiated Fricke solution was used as control.

The equation for the absorbance dose in the Fricke dosimetric solution is

$D = ɖA. NA/(\rho ɖ G\varepsilon d)$…….. (1)

Where,

D is the absorbed dose (Gy),

ɖA is the change in absorbance at 303 nm and 25° C (dimensionless),

ɖA = Ai— A0, where Ai and A0 are the absorbance's of the irradiated and non-irradiated solution, respectively,

NA is Avogadro's number (6.02 × 1023 mol^{-1}),

ρ is the density of the dosimetric solution (1.024 × 103 kg/m^3),

G is the radiation chemical yield of Fe3+ ions (9.74× 1017 molecules/j) (This G value is vaild for electrons or photons in the energy range 0.5–16 MeV at absorbed dose rates of less than 2 × 107 Gy/s), ε is the molar linear absorption coefficient (at 303 nm and 25°C) as measured for the particular spectrophotometer (with a nominal value of 219 m2/mol), and d is the optical path length in quartz cells, usually d = 0.01 m.

For irradiation and absorption measurement temperature of 25°C, with a 1.0 cm path length cuvette, and using the values of e and G given above, equation (1) reduces to

$$DFricke(Gy) = 278 x ɖA. \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (2)$$

The Fricke dosimetry system is primarily used in the experiment for dose mapping and determination of dose rate.

After selection of sample position and dose rate by Fricke dosimeters, Amber Perspex dosimeter was used for determination of delivered irradiation dose. The AmberPerspex dosimeter was prepared by Harwell Laboratory, Oxford shire, United Kingdom. In this experiment, Amber Perspex Dosimeter Type 3042 was used which has a dose detection range between 1 to 30 kGy. Amber 3042 are made from radiation-sensitive poly-methylmethacrylate (PMMA) in the form of optically transparent pieces individually sealed in laminate sachets. They darken when irradiated, and the radiation -induced darkening, accurately measurable by means of a spectrophotometer, is a function of the radiation dose absorbed (Glover and Plested 1993). The absorbance (A1) is measured at 603 nm or 651nm. The thickness (T1) of the dosimeter is also measured specific absorbance (A1/T1) is calculated and the determination delivered dose is done by using pre-existing graph /chart produced by Harwell Laboratory (Glover and Plested 1993).

After deliver dose determined, bottles for rice samples and packets of bread samples received 1 kGy, 3 kGy, 5 kGy, 7 kGy, 8 kGy, and 9 kGy gamma radiation.

3.7: Statistical Analysis:

Each data point represented three different experiments. Obtained data was analyzed using the Microsoft Excel program (Redmond, Washington DC, USA). Significant differences in plate count data were established by the least -significant difference ($p<0.05$) at the 5% level of significance.

4. Results

4.1. Disinfection of *B. cereus* KTCC-11204 in fried rice:

B. cereus KTCC-11204 was introduced into fried rice samples and the samples were irradiated by gamma radiation using cobalt-60 at 1 kGy, 3 kGy, 5 kGy, 7 kGy, 8 kGy and 9 kGy to determine complete removal of the strain. KTCC-11204 in fried rice samples showed significant decline in number (expressed in log cfu/gm) after gamma irradiation treatment. At 1 kGy radiation dose, the colony number of the KTCC-11204 was 5.02 log cfu/gm but at 3 kGy radiation dose, the number was significantly declined to 4.70 log cfu/gm (**figure 10**). While, at 5 kGy and 7 kGy, the numbers of KTCC-11204 colonies were found to be 3.57 log cfu/gm and 2.54 log cfu/gm, respectively. On the other hand, complete reduction of KTCC 11204 was obtained at 8 kGy.

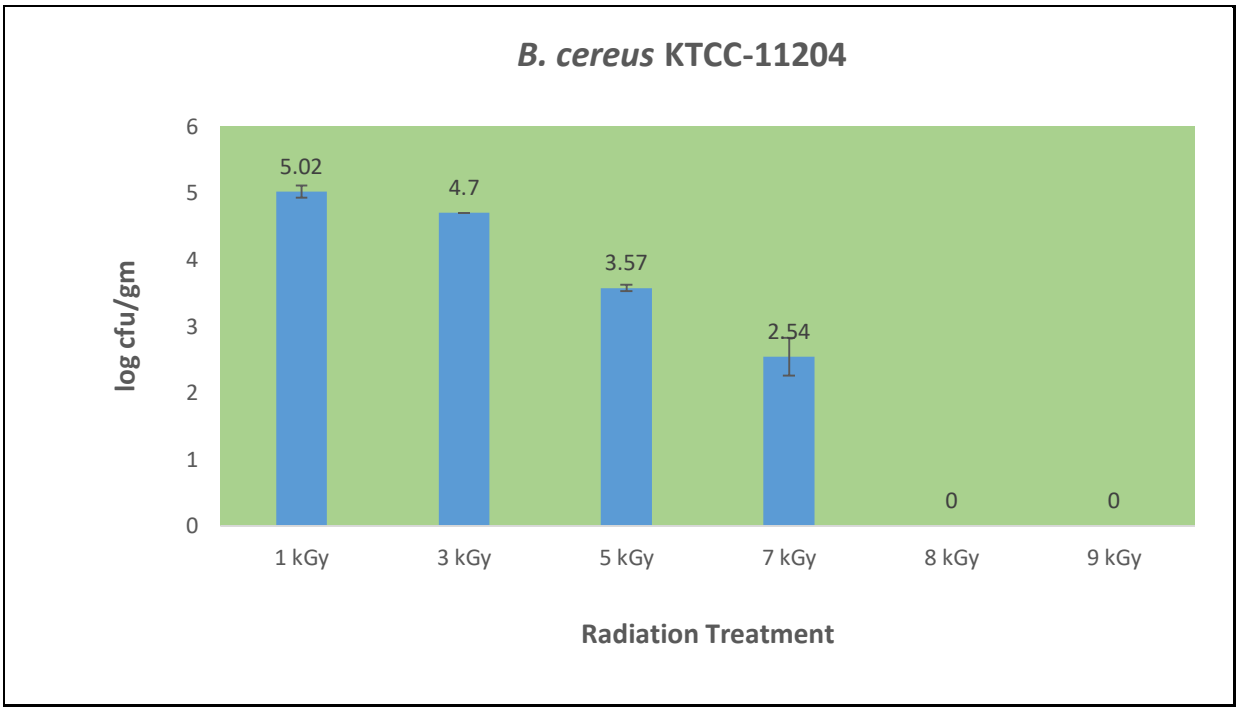

Figure 10: Disinfection of *B. cereus* KTCC-11204 in fried rice using gamma radiation.

4.2. Disinfection of *B. cereus* KTCC-400935 in fried rice:

The strain KTCC-400935 was mixed into fried rice and the samples were gamma irradiated using cobalt-60 at different doses (1 kGy, 3 kGy, 5 kGy, 7 kGy, 8 kGy and 9 kGy) to achieve the complete disinfection. At 1 kGy radiation dose the colony number of KTCC-400935 was 3.98 log cfu/gm but at 3 kGy radiation dose the number was significantly declined to 3.19 log cfu/gm (**Figure 11**). While, at radiation dose 5 kGy and 7 kGy, the numbers were found to be 2.65 log cfu/gm and 2.00 log cfu/gm, respectively. Importantly, for strain KTCC-400935 in fried rice a complete reduction was obtained at radiation dose 8 kGy.

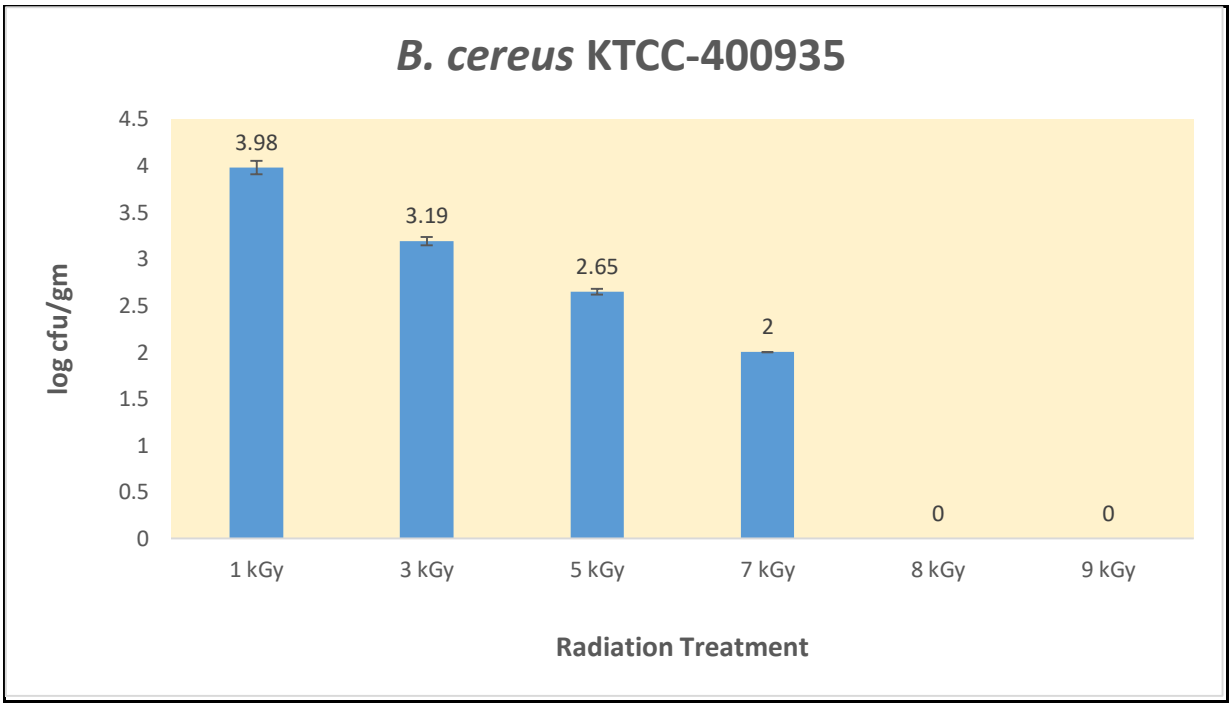

Figure 11: Disinfection of *B. cereus* KTCC-400935 in fried rice using gamma radiation.

4.3. Disinfection of *B. cereus* KTCC-11204 in Pasta:

The strain KTCC-11204 was inoculated into pasta and the samples were irradiated by gamma radiation using cobalt-60 at different doses (1 kGy, 3 kGy, 5 kGy, 7 kGy, 8 kGy and 9 kGy) to determine to a complete elimination. Data showed that KTCC-11204 in pasta samples were significantly decreased with an increasing radiation doses (**figure 12).** For instance, at 1 kGy the colony number of KTCC-11204 was found to be 6.70 log cfu/gm but at 3 kGy the number was significantly declined to 5.14 log cfu/gm. While, at 5 kGy and 7 kGy, the numbers were decreased to 4.24 log cfu/gm and 3.96 log cfu/gm, respectively. Notably, for strain KTCC-11204 in pasta a complete reduction was obtained at radiation dose 8 kGy.

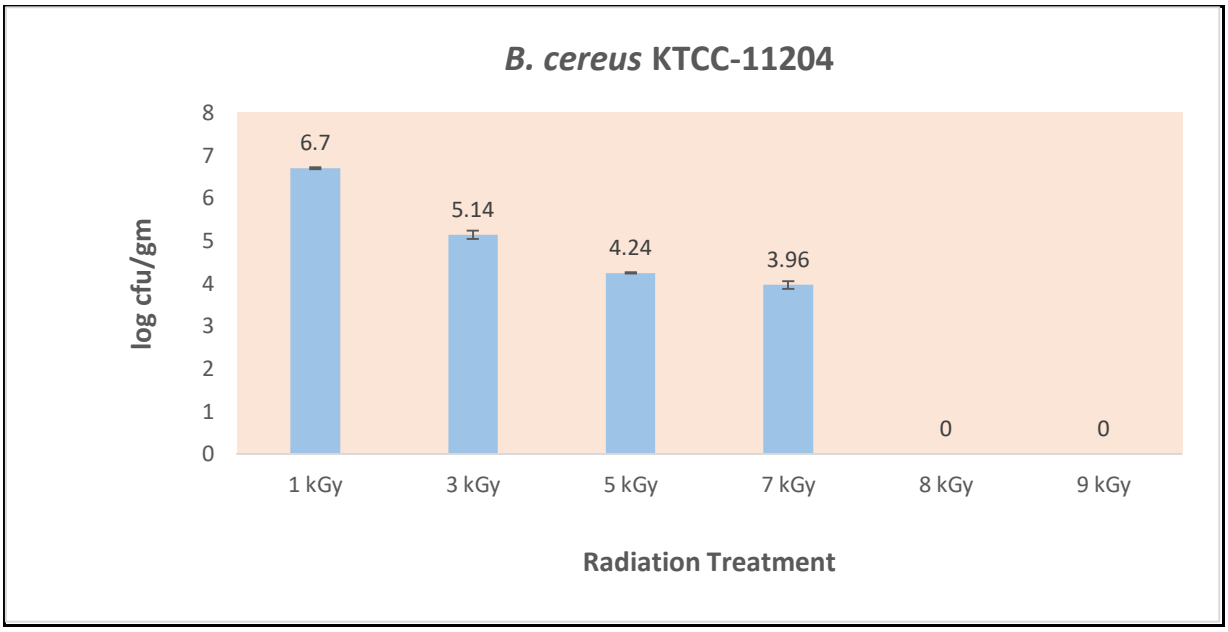

Figure 12: Disinfection of *B. cereus* KTCC-11204 in pasta using gamma radiation.

4.4. Disinfection of *B. cereus* KTCC-400935 in Pasta:

The strain KTCC-400935 was inoculated into the pasta and the samples were gamma irradiated by using cobalt-60 at different doses (1 kGy, 3 kGy, 5 kGy, 7 kGy, 8 kGy and 9 kGy) to achieve completely reduction of KTCC-400935. Data showed at 1 kGy dose the colony number of KTCC-400935 was 5.13 log cfu/gm but at 3 kGy radiation dose the number was significantly declined to 4.13 log cfu/gm (**figure 13**). While at 5 kGy and 7 kGy, the numbers of colonies were decreased 3.33 log cfu/gm and 3.25 log cfu/gm respectively. On the other hand, complete reduction can be obtained at 8 kGy.

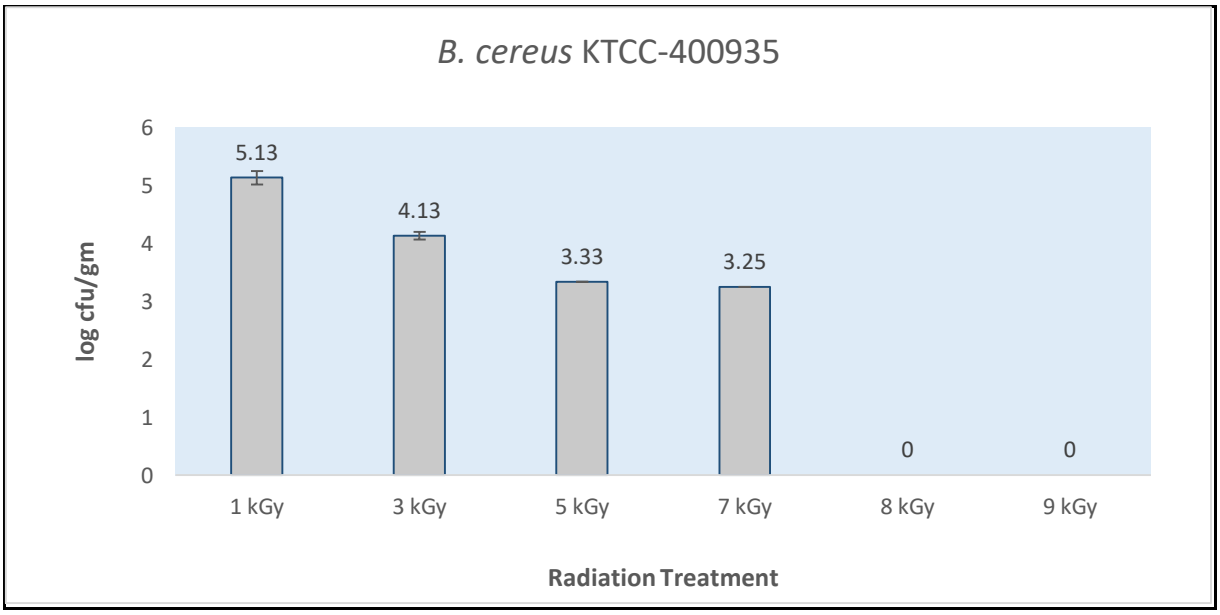

Figure 13: Disinfection of *B. cereus* KTCC-400935 in pasta using gamma radiation.

5. Discussion

At present, fried rice and pasta are the most popular dish among young generation in Bangladesh. In some Asian countries, small restaurants, street vendors and traveling hawkers specialize in serving fried rice. Fried rice is easily contaminated by *B. cereus* and most of the *B. cereus* related symptoms were reported after *B. cereus* contaminated fried rice ingestion. *B. cereus* is a toxin-producing bacteria that is one of the most common causes of food poisoning, also called "fried rice syndrome." Pasta is another food which is also easily contaminated by *B. cereus*. Both of fried rice and pasta can be contaminated by *B. cereus* easily because of unhygienic way of preparation. As fried rice is more popular and common than pasta among general people, so the study was first taken on fried rice sample and later on pasta sample.

B. cereus numbers in fried rice were completely reduced from 8.00 log cfu/gm after using gamma radiation. However, in the report of California department of health services (CDHS) stated that *B. cereus* counts remained in rice after 1 kGy and 3 kGy gamma radiation treatments were not safe for human consumption. The CDHS recommended for safe food consumption, *B. cereus* count should be less than 3 log cfu/gm. But at 5 kGy radiation dose treatment, the counts of *B. cereus* strain KTCC-11204 was also above 3 log cfu/gm that was 3.57 log cfu/gm. At 7 kGy radiation dose *Bacillus cereus* counts was below the effective range at 3 log cfu/gm which was 2.47 log cfu/gm.

Total elimination of *B. cereus* KTCC-11204 and *B. cereus* KTCC-400935 were observed at the 8 kGy radiation dose in both cases. According to Food and Agricultural Organization (FAO) and World Health Organization (WHO) (1998), gamma radiation doses above 10 kGy should not be applied to food matrices because of intoxicate the food materials; the getting result was found within the safety limit of gamma radiation. For the fried rice, total elimination is not necessary for safety but precautions should be taken. Hayashi et al. (1998) reported that the average doses of electrons necessary to reduce APCs of brown (husked) rice to levels lower than 10 cfu/gm or 1 log cfu/gm were 10 to 15 kGy, while gamma rays at 7.5 kGy produced the same microbial reduction [71]. Sarrias et al. (2002) stated that 7.5 kGy dose of electron beams reduced the microbial load to levels lower than 10 cfu/gm in husked rice inoculated approximately with 10^4 spores of *B. cereus* per gram [72].

In the study, we observed that but completely disinfection of *B. cereus* KTCC-11204 and *B. cereus* KTCC-400935 were obtained at the 8 kGy radiation dose in both cases. A fatal case due to liver failure after the consumption of a pasta based dish was reported by Dierick et al. (2005), demonstrating the possibility of extreme consequences of the emetic syndrome [73]. As reported by the Food Safety Authority of Ireland (FSAI, 2016), the emetic toxin can be produced by *B. cereus* strains in which vegetative cell counts are at least 10^5 cfu/gm or 5 log cfu/gm, whereas enterotoxins that cause the diarrheal syndrome are usually produced by *B. cereus* loads exceeding 10^6 cfu/gm or 6 log cfu/gm and further toxins are synthesized by the pathogen in the small intestine ofthe host. In this experiment showed that in pasta samples *B. cereu*s KTCC-11204 can stay easily above 6 log cfu/gm after 1 kGy radiation treatment, so this strain can easily produce enterotoxins in pasta and that will be very harmful to human.

In this study showed that the growth rates and the counts of the *B. cereus* of pasta samples were higher than fried rice samples in both types of strains. This result indicates that carbohydrate with spices environment are more potential and suitable for growth of *B. cereus*.

In conclusion the present study demonstrated that different *B. cereus* strains counts were different in same and different food matrix. The counts were also varied at same dose of gamma radiation. But after a certain doses the number of colonies of *B. cereus* were not found and complete elimination was obtained.

6. References

1. Kotiranta A, Lounatmaa K, Haapasalo M. Epidemiology and pathogenesis of *B. cereus* infections. Microbes Infect. 2000;2:189–198.

2. Hoffmaster, A., Hill, K., Gee, J., Marston, C., De, B., Popovic, T., Sue, D., Wilkins, P., Avashia, S., Drumgoole, R., Helma, C., Ticknor, L., Okinaka, R., and Jackson, J. "Characterization of *Bacillus cereus* Isolates Associated with Fatal Pneumonias: Strains Are Closely Related to *Bacillus anthracis* and Harbor *B. anthracis* Virulence." Journal of Clinical Microbiology. 2006. Volume 44(9). p. 3352-3360.

3. *"Bacillus cereus."* United States Food and Drug Administration, Center for food safety and applied nutrition (FDA). Accessed August 18, 2007.

4. Vilain, S., Luo, Y., Hildreth, M., and Brozel, V. "Analysis of the Life Cycle of the Soil Saprophyte *Bacillus cereus* in Liquid Soil Extract and in Soil." Applied Environmental Microbiology. 2006. Volume 72(7). p. 4970–4977.

5. Amano, K., Hazama, S., Akarari, Y., Ito, E. "Isolation and Characterization of Structural Components of *Bacillus cereus* AHU 1356 Cell Walls." European Journal of Biochemistry. (1977). Volume 75 (2). p. 513–522.

6. Severin, A., Tabei, K., Tomasz, A. "The structure of the cell wall peptidoglycan of *Bacillus cereus* RSVF1, a strain closely related to *Bacillus anthracis*." Microbial Drug Resistance. 2004. Volume 10(2). p. 77-82.

7. Mignot, T., Denis, B., Couture-Tosi, E., Kolsto, A., Mock, M., Fouet, A. "Distribution of S-layers on the surface of *Bacillus cereus* strains: phylogenetic origin and ecological pressure." Environmental Microbiology. 2001. Volume 3(8). p. 493–501.

8. Senesi, S., Celandroni, F., Salvetti, S., Beecher, D., Wong, A., and Ghelardi, A. "Swarming motility in *Bacillus cereus* and characterization of a *fliY* mutant impaired in swarm cell differentiation." Microbiology. 2002. Volume 148. p. 1785-1794.

9. Duport, C., Zigha, A., Rosenfeld, E., and Schmitt, P. "Control of Enterotoxin Gene Expression in *Bacillus cereus* F4430/73 Involves the Redox-Sensitive ResDE Signal Transduction System." Journal of Bacteriology. 2006. Volume 188. p. 6640–6651

10. Mols, M., de Been, M., Zwietering, M., Moezelaar, R., Abee, T. "Metabolic capacity of *Bacillus cereus* strains ATCC 14579 and ATCC 10987 interlinked with comparative genomics." Environmental Microbiology. 2007.

11. Mikkola, Raimo (2006). *Food and Indoor Air Isolated Bacillus Non-Protein Toxins: Structures, Physico-Chemical Properties and Mechanisms of Effects on Eukaryotic Cells* (PDF) (Thesis). University of Helsinki. p. 12. ISBN 952-10-3549-8. Archived (PDF) from the original on 9 July 2019. Retrieved 24 October 2015.

12. Rasko, D., Altherr, M., Han, C., and Ravel, J. "Genomics of the *Bacillus cereus* group of organisms." FEMS Microbiology Reviews. 2005. Volume 29(2). p.303-329.

13. "*Bacillus cereus.*" NCBI website. Accessed on August 18, 2007.

14. Rasko, D., Ravel, J., Okstad, O. A., Helgason, E., Cer, R., Jiang, L., Shores, K. A., Fouts, D., Tourasse, N., Angiuoli, S., Kolonay, J., Nelson, W., Kolsto, A, Fraser, C., and Read, T. D. "The genome sequence of *Bacillus cereus* ATCC 10987 reveals metabolic adaptations and a large plasmid related to *Bacillus anthracis* pXO1" Nucleic Acids Research. 2004. Volume 32(3). p. 977–988.

15. Wijnands, L., Dufrenne, J., Zwietering, M. H., and Leusden, F. "Spores from mesophilic *Bacillus cereus* strains germinate better and grow faster in simulated gastro-intestinal conditions than spores from psychrotrophic strains." International Journal of Food Microbiology. 2006. Volume 112. Issue 2. p. 120-128.

16. Han, C., Xie, g., Challacombe, J., Altherr, M., Bhotika, S., Bruce, D., Campbell, D., Campbell, M., Chen, J., Chertkov, O., Cleland, C., Dimitrijevic, M., Doggett, N., Fawcett, J., Glavina, T., Goodwin, L., Hill, K., Hitchcock, P., Jackson, P., Keim, P., Kewalramani, A., Longmire, J., Lucas, S., Malfatti, S., McMurry, K., Meincke, L. J., Misra, M., Moseman, B. L., Mundt, M., Munk, C., Okinaka, R. T., Parson-Quintana, B., Reilly, L. P., Richardson, P., Robinson, D. L., Rubin, E., Saunders, E., Tapia, R., Tesmer, J. G., Thayer, N., Thompson, L. S., Tice, H., Ticknor, L., Wills, P., Brettin, T., and Gilna, P. "Pathogenomic Sequence Analysis of *Bacillus cereus* and *Bacillus thuringiensis* Isolates Closely Related to *Bacillus anthracis."* Journal of Bacteriology. 2006. Volume 188(900). p. 3382–3390.

17. World Health Organization. High-Dose Irradiation: Wholesomeness of Food Irradiated With Doses Above 10 kGy. Report of a Joint FAO/IAEA/WHO Study Group. Geneva, Switzerland: World Health Organization; 1999. WHO Technical Report Series No. 890

18. C.M. Deeley, M. Gao, R. Hunter, D.A.E. Ehlermann, The development of food irradiation in the Asia Pacific, the Americas and Europe; tutorial presented to the International Meeting on Radiation Processing, Kuala Lumpur, 2006.

19. Fellows, P.J. (2018). *Food Processing Technology: Principles and Practices*. Elsevier. pp. 279–280.

20. EFSA (European Food Safety Authority), (2011). Statement summarizing the conclusions and recommendations from the opinions on the safety of irradiation of food adopted by the BIOHAZ and CEF panels, The EFSA J., Vol. 9, no. 4, pp. 2107.

21. Scott Smith, J.; & Suresh, P. (2004). Irradiation and food safety, Food tech., irradiation & food safety. Vol. 58, no. 11, pp. 48-55.

22. Koopmans, M. & Duizer, E. (2004). Food borne viruses: an emerging problem, J. Food Microb., Vol. 90, pp. 23-24.

23. Ahari, M. H.; Fathollahi, H.; Motamedi, F. & Mirmajlessi, S. M. (2010). Food irradiation: Applications, public acceptance and global trade: a review. African J. Biotech., Vol. 9, no. 20, pp. 2826-2833.

24. Fan, X. T. & Sommers, C. H. (2006). Effect of gamma radiation on furan formation in ready-to-eat products and their ingredients, J. Food Sci., Vol. 71, pp. C407-C412.

25. Beecher D.J. Wong A.C.L. (1997) Tripartite hemolysin BL from *Bacillus cereus*. *Hemolytic analysis of component interaction and model for its characteristic paradoxical zone phenomenon. J. Biol. Chem.* 272, 233–239.

26. Granum P.E. (1994) *Bacillus cereus* and its toxins. *J. Appl. Bacteriol. Symp. Suppl.* 76, 61S–66S.

27. Kramer J.M. Gilbert R.J. (1989) *Bacillus cereus* and other Bacillus species. In: *Foodborne Bacterial Pathogens* (Doyle M.P., Ed.), pp. 21–70. Marcel Dekker, New York.

28. Granum P.E. (1997) *Bacillus cereus*. In: *Fundamentals in Food Microbiology* (Doyle M. Beuchat L).

29. Agata N.Mori M. Ohta M. Suwan S. Ohtani I. Isobe M. (1994) A novel dodecadepsipeptide, cereulide, isolated from *Bacillus cereus* causes vacuole formation in HEp-2 cells. FEMS Microbiol. Lett. 121, 31–34.

30. EFSA (2012) The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2010. EFSA J 10: 2597.

31. Fricker M, Reissbrodt R & Ehling-Schulz M (2008) Evaluation of standard and new chromogenic selective plating media for isolation and identification of Bacillus cereus. Int J Food Microbiol 121: 27–34.

32. Ehling-Schulz M, Svensson B, Guinebretiere MH et al. (2005) Emetic toxin formation of *Bacillus cereus* is restricted to a single evolutionary lineage of closely related strains. Microbiology 151: 183–197.

33. Pirhonen TI, Andersson MA, Jaaskelainen EL, SalkinojaSalonen MS, Honkanen-Buzalski T & Johansson TML (2005) Biochemical and toxic diversity of *Bacillus cereus* in a pasta and meat dish associated with a food-poisoning case. Food Microbiol 22: 87–91.

34. Samapundo S, Heyndrickx M, Xhaferi R & Devlieghere F (2011) Incidence, diversity and toxin gene characteristics of *Bacillus cereus* group strains isolated from food products marketed in Belgium. Int J Food Microbiol 150: 34–41.

35. Ankolekar C, Rahmati T & Labbe RG (2009) Detection of toxigenic *Bacillus cereus* and *Bacillus thuringiensis* spores in US rice. Int J Food Microbiol 128: 460–466.

36. EFSA, 2005. EFSA panel on biological hazards. opinion of the scientific panel on biological hazards on Bacillus cereus and other *Bacillus spp*. in foodstuffs. EFSA J. 175, 1–48.

37. Granum PE & Lund T (1997) Bacillus cereus and its food poisoning toxins. FEMS Microbiol Lett 157: 223–228. Granum PE, Brynestad S, O'Sullivan K & Nissen K (1993) Enterotoxin from *Bacillus cereus*: production and biochemical characterization. Neth Milk Dairy J 47: 63–70.

38. Agata N, Ohta M & Yokoyama K (2002) Production of *Bacillus cereus* emetic toxin (cereulide) in various foods. Int J Food Microbiol 73: 23–27

39. Shinagawai K, Ueno Y, Hu D, Ueda S & Sugii S (1996) Mouse lethal activity of a HEp-2 vacuolation factor, cereulide, produced by Bacillus cereus isolated from vomiting-type food poisoning. J Vet Med Sci 58: 1027–1029.

40. Rajkovic A, Uyttendaele M, Vermeulen A, Andjelkovic M, FitzJames I, in't Veld P, Denon Q, Verhe R & Debevere J (2008) Heat resistance of *Bacillus cereus* emetic toxin, cereulide. Lett Appl Microbiol 46: 536–541.

41. Turnbull PC, Kramer JM, Jorgensen K, Gilbert RJ & Melling J (1979) Properties and production characteristics of vomiting, diarrheal, and necrotizing toxins of *Bacillus cereus*. Am J Clin Nutr 32: 219–228.

42. Granum PE, Brynestad S, O'Sullivan K & Nissen K (1993) Enterotoxin from *Bacillus cereus*: production and biochemical characterization. Neth Milk Dairy J 47: 63–70.

43. Wijnands LM, Dufrenne JB & van Leusden FM (2005) RIVM report 250912003/2005 *Bacillus cereus*: characteristics, behaviour in the gastro-intestinal tract, and interaction with Caco-2 cells. Report from RIVM (National Institute for Public Health and the Environment "Rijksinstituut voor Volksgezondheid en Milieu" in the Netherlands).

44. Ceuppens S, Rajkovic A, Hamelink S, Van de Wiele T, Boon N & Uyttendaele M (2012a) Enterotoxin production by *Bacillus cereus* under gastrointestinal conditions and their immunological detection by commercially available kits. Foodborne Pathog Dis, 9: 1130–1136.

45. Bottone, E.J., 2010. *Bacillus cereus*, a volatile human pathogen. Clin. Microbiol. Rev. 23, 382–389.

46. Garofalo, C., Osimani, A., Milanović, V., Taccari, M., Cardinali, F., Aquilanti, L., Riolo, P., Ruschioni, S., Isidoro, N., Clementi, F., 2017. The microbiota of marketed processed

47. Osimani, A., Garofalo, C., Milanović, V., Taccari, M., Cardinali, F., Aquilanti, L., Pasquini, M., Mozzon, M., Raffaelli, N., Ruschioni, S., Riolo, P., Isidoro, N., Clementi, F., 2017. Insight into the proximate composition and microbial diversity of edible insects marketed in the European Union. Eur. Food Res. Technol. 243, 1157–1171.

48. EFSA, 2005. EFSA panel on biological hazards. opinion of the scientific panel on biological hazards on Bacillus cereus and other Bacillus spp. in foodstuffs. EFSA J. 175, 1–48.

49. Ghelardi, E., Celandroni, F., Salvetti, S., Barsotti, C., Baggiani, A., Senesi, S., 2002. Identification and characterization of toxigenic *Bacillus cereus* isolates responsible for two food-poisoning outbreaks. FEMS Microbiol. Lett. 208 (1), 129–134.

50. Fricker, M., Messelhäusser, U., Busch, U., Scherer, S., Ehling-Schulz, M., 2007. Diagnostic real-time PCR assays for the detection of emetic *Bacillus cereus* strains in foods and recent food-borne outbreaks. Appl. Environ. Microbiol. 73 (6), 1892–1898.

51. Slaten D.D., Oropeza R.I., Werner S.B., An outbreak of *Bacillus cereus* food poisoning – are caterers supervised sufficiently, Public Health Rep. 107 (1992) 477–480.

52. Aas N., Gondrosen B., Langeland G., Norwegian food control authority´s report on food associated diseases in 1990, SNT-report 3, Oslo, 1992.

53. Pan T.M., Chiou C.S., Hsu S.Y., Huang H.C., Wang T.K., Chiu S.I., Yea H.L., Lee C.L., Food-borne disease outbreaks in Taiwan, 1994, J. Formosan Med. Assoc. 95 (1996) 417–420.

54. Ceuppens S, Rajkovic A, Heyndrickx M, Tsilia V, van de Wiele T, Boon N & Uyttendaele M (2011) Regulation of toxin production by *Bacillus cereus* and its food safety implications. Crit Rev Microbiol 37: 188–213.

55. Jaaskelainen EL, Haggblom MM, Andersson MA, Vanne L & Salkinoja-Salonen MS (2003) Potential of *Bacillus cereus* for producing an emetic toxin, cereulide, in bakery products: quantitative analysis by chemical and biological methods. J Food Prot 66: 1047–1054.

56. Rajkovic A, Uyttendaele M, Ombregt SA, Jaaskelainen E, Salkinoja-Salonen M & Debevere J (2006b) Influence of type of food on the kinetics and overall production of *Bacillus cereus* emetic toxin. J Food Prot 69: 847–852.

57. Kim JB, Kim JM, Kim SY, Kim JH, Park YB, Choi NJ & Oh DH (2010) Comparison of enterotoxin production and phenotypic characteristics between emetic and enterotoxic *Bacillus cereus*. J Food Prot 73: 1219–1224.

58. Jaaskelainen EL, Haggblom MM, Andersson MA & SalkinojaSalonen MS (2004) Atmospheric oxygen and other conditions affecting the production of cereulide by *Bacillus cereus* in food. Int J Food Microbiol 96: 75–83.

59. Rajkovic A, Uyttendaele M, Deley W, Van Soom A, Rijsselaere T & Debevere J (2006a) Dynamics of boar semen motility inhibition as a semi-quantitative measurement of *Bacillus cereus* emetic toxin (Cereulide). J Microbiol Methods 65: 525–534.

60. Haggblom MM, Apetroaie C, Andersson MA & SalkinojaSalonen MS (2002) Quantitative analysis of cereulide, the emetic toxin of *Bacillus cereus*, produced under various conditions. Appl Environ Microbiol 68: 2479– 2483.

61. Clavel T, Carlin F, Lairon D, Nguyen-The C & Schmitt P (2004) Survival of *Bacillus cereus* spores and vegetative cells in acid media simulating human stomach. J Appl Microbiol 97: 214–219.

62. Clavel T, Carlin F, Dargaignaratz C, Lairon D, Nguyen-The C & Schmitt P (2007) Effects of porcine bile on survival of *Bacillus cereus* vegetative cells and haemolysin BL enterotoxin production in reconstituted human small intestine media. J Appl Microbiol 103: 1568–1575.

63. Ceuppens S, Uyttendaele M, Drieskens K, Rajkovic A, Boon N & Van de Wiele T (2012b) Survival of *Bacillus cereus* vegetative cells and spores during in vitro simulation of gastric passage. J Food Prot 75: 690–694.

64. Wijnands LM, Dufrenne JB, Rombouts FM, In't Veld PH & Van Leusden FM (2006a) Prevalence of potentially pathogenic *Bacillus cereus* in food commodities in The Netherlands. J Food Prot 69: 2587–2594.

65. Rahmati T & Labbe R (2008) Levels and toxigenicity of *Bacillus cereus* and Clostridium perfringens from retail seafood. J Food Prot 71: 1178–1185.

66. Kim HJ, Lee DS & Paik HD (2004) Characterization of *Bacillus cereus* isolates from raw soybean sprouts. J Food Prot 67: 1031–1035.

67. Park YB, Kim JB, Shin SW, Kim JC, Cho SH, Lee BK, Ahn J, Kim JM & Oh DH (2009) Prevalence, genetic diversity, and antibiotic susceptibility of *Bacillus cereus* strains isolated from rice and cereals collected in Korea. J Food Prot 72: 612–617.

68. Eglezos S, Huang BX, Dykes GA & Fegan N (2010) The prevalence and concentration of *Bacillus cereus* in retail food products in Brisbane, Australia. Foodborne Pathog Dis 7: 867–870.

69. Guinebretiere MH & Nguyen-The C (2003) Sources of *Bacillus cereus* contamination in a pasteurized zucchini puree processing line, differentiated by two PCR-based methods. FEMS Microbiol Ecol 43: 207–215.

70. Guinebretiere MH, Girardin H, Dargaignaratz C, Carlin F & Nguyen-The C (2003) Contamination flows of *Bacillus cereus* and spore-forming aerobic bacteria in a cooked, pasteurized and chilled zucchini puree processing line. Int J Food Microbiol 82: 223–232.

71. Hayashi, T., Okadome, H., Toyoshima, H., Todoriki, S., Ohtsubo, K., 1998. Rheological propertiesand lipid oxidation of rice decontaminated with low-energy electrons. J. Food Prot. 61, 73–77.

72. Sarrías, J.A., Valero, M., Salmerín, M.C., 2002. Enumeration, isolation and characterization of *Bacillus cereus* strains from Spanish raw rice. Food Microbiol., in press.

73. Dierick, K., Van Coillie, E., Swiecicka, I., Meyfroidt, G., Devlieger, H., Meulemans, A., Hoedemaekers, G., Fourie, L., Heyndrickx, M., Mahillon, J., 2005. Fatal family outbreak of *Bacillus cereus*-associated food poisoning. J. Clin. Microbiol. 43 (8), 4277–4279.

74. Ash C., Farrow J.A., Dorsch M., Stackebrandt E., Collins M.D., Comparative analysis of *Bacillus anthracis*, *Bacillus cereus*, and related species on the basis of reverse transcriptase sequencing of 16S rRNA, Int. J. System. Bacteriol. 41 (1991) 343–346.

Appendix

1. Nutrient Broth preparation:

Nutrient Broth is the liquid version of the solid medium. It is a classical meat infusion broth that is useful for the routine laboratory purposes.

Formula in g/L

Substances	Amount (g/L)
Meat extract	1.00
Yeast extract	2.00
Peptone	5.00
Sodium chloride	5.00

Final pH 7.4 ± 0.2 at 25ºC

Measured 0.8 g of nutrient broth and took it into 250 ml conical flask. Added 100 ml distilled water and dissolved the nutrient broth by shaking. Nutrient broth media was then sterilized at 121º C temperature for 15 minutes. *Bacillus cereus* were inoculated in cooled broth media and incubated for 24 hours.

2. Normal Saline solution preparation:

Normal Saline solution growth media provide isotonic medium for growth and dilution procedures.

Substances	Amount
Sodium chloride	0.9 g (0.9%)
Distilled Water	100 ml

Measured 100 ml distilled water in a 250 ml conical flask and dissolved 0.9 g sodium chloride into distilled water. Sterilized the normal saline solution at 121º C for one hour.