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Incidence of *Bacillus cereus* in Corn Snacks and its Control Using Gamma Radiation

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Abstract: Samples of 20 different commercial corn snacks products were collected from Egyptian market. Three replicates of each product were used to determine their bacterial burden including total mesophilic bacteria, spore formers and *Bacillus cereus* group. Total mesophilic counts ranged between $6.4x10^2$ to $8.6x10^6$ cfu/g, spore forming bacteria were less than 100 to $6.6x10^4$ cfu/g and *B. cereus* were detected in 14 products out of twenty(70%). Products encoded 9,11,13,18 and 20 showed highest *B. cereus* prevalence among spore formers (39.5, 26.5, 18.6, 13.6 and 12.5%). Upon exposing the five chosen samples to increasing doses of gamma rays significant decrease of the cereus group load correlating with initial counts (cfu/g) of each sample was observed. Dose levels 7, 8 & 9 kGy completely eliminated bacilli endospores contaminating corn snacks samples no.(18), (9&20) and (11&13), respectively. Thirty eight spore forming strains were chosen from MYP plates and differentiated into different species using staining and biochemical tests according to key of Bergey's manual. *B. cereus* was the most frequent species (21 isolates) representing 55.3% of total isolated bacteria. The 21 identified strains were screened for their virulence factors using agar diffusion method. *B. cereus* strains BC13 and BC37 were found to be the most potent isolates. Studying the effect of incubation temperatures on growth of both strains after different time intervals revealed that 35°C was the optimum temperature achieving maximum growth after nearly 24 h. Low and high incubation temperatures extended the lag phase of both strains, yet they were capable of increasing in number by about one log cycle after 24 h at 7°C and 36 h at 55°C. Screening for the virulence factors showed the capability of both strains to produce the three enzymes (hemolysin, phospholipase and protease enzymes) at all investigated incubation temperatures (7, 20, 35, 45 &55°C). The dose response curves of the two selected potent strains (BC13 & BC37) revealed their significant radio-resistance and the calculated D_{10} values were 2.9 and 2.8 kGy, respectively. Also, both strains were capable of producing their virulence factors even after being irradiated with doses up to 8 kGy.

Key words: Corn snacks, *Bacillus cereus,* virulence factors, gamma radiation, incubation temperature, incubation period.

INTRODUCTION

B. cereus is wide spread in the soil and food industry. It has a broad range of foods associated with infection including herbs, spices, milk, meat, raw and cooked vegetables, boiled or fried rice, vanilla sauce, custards, soups, ice cream and cereals. Contamination of food with *B. cereus* is often associated with food poisoning as a result of heat stable enterotoxins produced either in food or in intestines (Mckillip, 2000). There are two types of food illnesses associated with *B. cereus.* The most common is a diarrheal illness caused by heat labile toxin and an emetic illness caused by a heat stable toxin (Granum, 2001 and Schoeni and Wong, 2005). Spores of *B. cereus* are heat-resistant and can survive cooking and heat treatments of various foods. Heat resistance of spores is increased by high salt concentrations and mild or gradual heating. Thus, it could be said that one of the leading causes of food borne *Bacillus* infections and intoxications comes from the improper hot holding of prepared food items (Schneider *et al*., 2004). *B. cereus* is ubiquitous in the environment and preventing contamination of food with its spores is almost impossible.

Raw foods of plant origin are the major source of *B. cereus*. The widespread distribution of the organism, the ability of spores to survive dried storage and thermal resistance of spores, means that most ready-to-eat foods will contain *B. cereus* and will require control measures to prevent growth especially after cooking has eliminated other competing flora (Notermans and Batt, 1998).

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Contaminated food is a real threat to human welfare. Thus, a standard hygiene control system is needed in order to lower the level of contamination to a safe level. Using different preservation methods is limited by many factors, mainly nature of material to be decontaminated and availability of sterilizing means (White *et al*., 1996 and Brinston, 1995). Although the use of radiation for decontamination is not very old, it has found its way into many applications including food preservation. Ionizing radiation is highly damaging to biological system but very practical in inducing sterility without noticeable changes in the appearance or structure of irradiated materials (Tiliquin, 1991; O'Doherty, 1997 and SainzVidal *et al*., 1999).

The objective of this work was to investigate the incidence and frequency of *B. cereus* in corn snacks products in the Egyptian market. Studying the effect of different incubation temperatures on some virulent strains of food borne *B. cereus* and using gamma radiation for its elimination in corn snacks samples was also attempted.

MATERIALS AND METHODS

Collection of samples:

Replicates of corn snacks packets of twenty different commercial names were collected from the market in Zagazig Cairo Giza cities. Packets of the same commercial name were opened under aseptic conditions, thoroughly mixed and re-packed as 10gs weights in sterile polyethylene bags and heat sealed. The prepared corn snacks packets were stored in a cooling incubator until time of experiment.

Microbiological analysis:

10gs of the prepared corn snacks samples were homogenized in 90ml of sterile saline solution (0.85%NaCl). Decimal dilutions up to 10^{-8} were prepared to enumerate total mesophilic bacteria using pour plate technique and tryptone soya agar (TSA) medium (Oxoid, 1998).

Total spore forming bacteria were counted after placing the corn snacks homogenates in water bath at 75°C for 20 minutes using pour plate technique and TSA medium.

B. cereus were enumerated and isolated by surface spread of 0.1ml aliquots on surface of mannitol yolk polymxin agar (MYP) medium (APHA, 1992 and FDA, 2001). Suspected pink colonies growing on MYP agar plates are picked, purified and identified according to Krieg and Holt (1984) and Murray *et al*.(1999); Rhodehamel and Harmon (2001) and Todar (2005) using differential stains and biochemical tests.

Determination of virulence factors:

Produced hemolysin, lecithinase and protease enzymes were determined using agar well diffusion assay according to Reinheimer *et al*. (1990) and Misra and Kuila (1992). Wells in blood agar, egg yolk agar and casein agar plates were filled with 25µl aliquots of filter-sterilized (0.45µm pore size) *B. cereus* culture filtrates. Plates were incubated at desired temperature for 24 h.

Gamma irradiation process:

Previously prepared polyethylene bags containing 10gs corn snacks of different commercial products were exposed to increasing doses of gamma rays using ^{60}Co gamma cell (Indian) located at National Center for Radiation Research and Technology (NCRRT), Nasr City, Cairo, Egypt. Dose rate at time of experiment was 5.596kGy/h. Three replicates were prepared for each treatment.

Dose response curves:

Spore suspensions of both selected strains were prepared by inoculating loopfulls of growing cultures in tryptone soya broth (TSB) and incubated for 48 h. Bacterial cultures were centrifuged at 3000rpm. Supernatants were decanted and spores washed twice with sterile distilled water. Separated endospores were re-suspended in sterile saline solution and inoculum size was adjusted so that each ml contained ~10^8cfu. The prepared spore suspensions were subjected to dose levels 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 kGy (three replicates were tested for each dose). D_{10} values of the two most potent *B. cereus* strains (BC13 and BC37) were calculated according to WHO, (1981).

Effect of gamma irradiation on production of virulence factors:

1ml aliquots of irradiated spores of both selected strains were inoculated in TSB medium and incubated for 24 h. 25µl of culture filtrates were filter-sterilized and virulence factors were determined as mentioned before.

Statistical analysis:

Results obtained were statistically evaluated through SPSS for Windows version 11.0, t-value test, bivariate correlations coefficient (r), Coefficient of Deviation (C.D.) test, calculated Standard Deviation (S.D.) and the two-way analysis of variance (ANOVA) according to (Steel and Torie, 1980 and Davis, 1986).

RESULTS AND DISCUSSION

B. cereus has been isolated in several countries from a variety of foods including vegetables, puddings, sauces, milk, dairy products, fried and cooked rice (Schneider *et al*., 2004, Svensson *et al*., 2007 and FDA/CFSAN, 2007). Also, the prevalence of toxigenic stains of *B. cereus* in different starchy foods such as pulses and cereals, cereal meals, noodles, pasta, bread, mashed potatoes, infant cereal formulas and dehydrated potatoes has been extensively reported (Blakey and priest, 1980; Hanis *et a*l., 1988; Agata *et al*., 2002; Duc *et al.,* 2005; Shaheen *et al*., 2006 and King *et al*., 2007), thus it was important to evaluate the corn snacks products widely distributed and sold in Egypt.

Replicates of 20 different commercial products of corn snacks were collected from the Egyptian market in Cairo, Giza and Zagazig cities. The bacterial burden of the samples was determined including total mesophilic bacteria, total spore formers (at 75^{o}C) and *B.cereus* group (using MYP agar). Data in table (1) revealed that total mesophilic counts of the examined corn snacks samples ranged between $6.4x10^{2}$cfu/g and $5.3x10^{6}$ cfu/g while the spore formers ranged between ≤100 and $6.6x10^{4}$cfu/g. Obviously, fourteen samples out of twenty were contaminated with spores of bacteria related to the cereus group with counts ranging between 1.5x10 and $6.1x10^{3}$cfu/g and samples no. 9,11,13,18 and 20 recorded high frequency (%) of contamination (39.5, 26.5, 18.6, 13.6 and 12.5%, respectively).

The production and consumption of cereals based food is increasing and most often their raw materials may become rather contaminated with various hazardous microorganisms during harvesting, processing and packaging. It should be taken in consideration that most of these products are consumed either without additional heat treatment or mild heating (Blakey and Priest, 1980). For this reason it is highly desirable to reduce pathogenic microflora and gamma radiation may be a method of choice, particularly considering the destruction of nutrients by alternative heat decontamination or problems related to the use of food additives (Maxcy, 1982 and Campbell *et al*., 1986). The required doses for reasonable reduction of microbial load are usually more than 1 kGy (Ingram and Farkas, 1977). Dose levels up to 10 kGy are effective in microbial decontamination of cereal meals including corn wheat and oat meals and do not adversely affect the sensory and nutritional quality of such foods (Hanis *et al*., 1988). Tauxe (1997 &2001) reported the efficiency of gamma irradiation in protecting the public from food borne infections with doses that vary with the specific pathogen and circumstances of the specific food. He also observed that the effect of irradiation on food itself is usually minimal at doses up to 7.5 kGy.

The five corn snacks samples contaminated with high percentages of bacilli related to the cereus group were subjected to increasing doses of gamma radiation (1, 2, 3, 4, 5, 6, 7, 8, 9&10 kGy). Obvious correlation was observed between the initial counts of bacterial spores contaminating the products and the required dose for their complete elimination (table 2) whereas samples no. 11&13 harbored highest counts ($6.1x10^{3}$ and $1.6x10^{3}$ cfu/g) and the eliminating dose was 9 kGy. Meanwhile, initial counts of samples no.9&20 were $8.3x10^{2}$ and $3.3x10^{2}$ cfu/g, respectively and their eliminating dose was 8 kGy and in case of sample no. 18 the dose level 6 kGy was enough to eliminate the bacilli spores (table 2).

Thirty eight bacterial isolates were randomly chosen and picked up from MYP plates used for enumeration of bacteria related to the cereus group. The purified bacterial strains were further on identified and differentiated into their species according to key of Bergey's manual (Krieg and Holt, 1984). Table (3) shows the incidence and frequency of *Bacillus cereus* species in the five mostly contaminated corn snacks samples. The average frequency of *B. cereus* in the five examined corn snacks samples was 55.3% of total isolated bacteria related to cereus group. Accordingly, several reports had confirmed the high frequency of *B. cereus* species within a wide variety of raw and processed food (Agata *et a*l., 2002; Duc *et al*., 2005; Shaheen *et al*., 2006; King *et al*., 2007).

B. cereus currently attracted increasing attention due to its capability of producing a range of membrane active, tissue degrading enzymes and enterotoxins (Drobniewski, 1993; Schoeni and Wong, 2005 and Lund and Granum, 1997). *B. cereus* strains were observed to produce an emetic and diarrhoeal enterotoxins beside other virulence factors including phospholipase protease and hemolysins, one of which cereolysin is a thiol activated hemolysin. These virulence factors may be contributed to enteric and non enteric diseases (Drobniewski, 1993). *B. cereus* emetic toxin has been associated with life threatening acute conditions such as fulminant liver

failure and rhabdomylosis (Mahler *et al.,* 1997 and Yokoyama *et al.,* 1999). This toxin is unique among enterotoxins since it is resistant to proteolytic degradation, pH extremes and elevated temperatures surviving 121°C for 90 minutes (Granum and Lund, 1997).

Data in table (4) revealed that strains BC13 and BC37 were the most potent strains among the 21 identified *B. cereus* isolates concerning their virulence factors (hemolysin, lecithinase and protease) determined by well agar diffusion assay as zone diameters (mm) on blood agar, egg yolk agar and casein agar plates.

B. cereus is ubiquitously distributed aerobic spore formers that tolerate adverse environmental conditions better than other bacterial pathogens (Rowan, 1996). Several studies have shown that *B. cereus* strains are capable of producing enterotoxins implicated in out breaks of food related illnesses under wide range of temperatures even in refrigerated food (Rowan and Anderson, 1998, Fermanian *et al.,* 1997; Finaly *et al.,* 2000) Growth of both isolates (BC13 and BC37) was determined after different incubation periods (4, 8, 12, 16, 20, 24, 28, 32, 36 and 40h) and at different incubation temperatures (7, 20, 35, 45 and 55°C). Fig (1a&b) shows that maximum constant growth of both strains was achieved after 28 h at 35°C. Meanwhile, very low temperature (7°C) and high temperature (55°C) extended the lag phase and an increase of ~ 1 log cycle in the viable counts (cfu /g) of BC13 and BC37 was observed after 24 and 28 h, respectively.

The two potent strains BC13 and BC37 were screened for their virulence factors at different incubation temperatures (7, 20, 35, 45 and 55°C) after 24 h using agar diffusion method (table 5). The correlation between produced virulence factors and variation of incubation temperature was well observed where maximum diameters were recorded in case of incubation at 35°C followed by 45°C and 20 °C, while in case of low incubation temperature (7°C) a significant decrease in the diameter of formed zones of all tested factors was observed but still their virulence is well recognizable. Sharp decrease in zone diameters of the three virulence factors was recorded for both strains when incubated at 55°C. These results assess the risk of consumption of corn snacks harboring *B. cereus* spores capable of growing and producing its virulence factors over a wide range of incubation temperatures (7 to 55°C). This observation reinforces the need of using a method to eliminate its presence in these products.

Fermanian *et al*. (1997) observed the capability of *B. cereus* strains F4433/73 and F4581/76 to produce diarrheal toxin during its exponential phase at 32°C as well as at 10°C. Rowan and Anderson (1998) isolated *B. cereus* from milk based infant formulae capable of growing and producing enterotoxins in temperature range 4-8°C. Finlay *et al*. (2000) reported that 53% of the examined strains of *B. cereus* grew at 7°C and five strains produced emetic toxin at 12°C after 4days of incubation.

Radioresistance of *Bacillus spp*. had been studied by several authors. The calculated D_{10} values of *B. pumilus*, *B. cereus* and *B. botulinum* dried on glass fibers were 1.8, 1.1 and 2.2 kGy, respectively (Ito, 2001). Chawla *et al*.(2003) reported that D_{10} value of *B. cereus* in semi-dried sea food products was 0.64kGy, while in marinated beef ribs was 0.66 kGy (Jo *et al*., 2004). D_{10} values of different strains of *B. cereus* isolated from Spanish raw rice ranged between 2.07-2.68 kGy (Sarrias *et al.*, 2003). Also, Zahran *et al*. (2008) observed high resistance of a toxigenic strain isolated from chicken luncheon with D_{10} value 1.9 kGy.

Spore suspensions of the two strains BC13 and BC37 were prepared and exposed to increasing doses of gamma radiation (1, 2, 3, 4, 5, 6, 7, 8, 9 & 10 kGy). The viability of spores was determined as log no. of cfu/ml and survival curves were plotted in fig (2). Calculated D_{10} values were 2.9 and 2.8 kGy for BC13 and BC37 strains, respectively reflecting obvious radioresistance of *B. cereus* spores.

The capability of irradiated spores to produce its virulence factors after treatment with increasing doses of gamma radiation (1, 2, 4, 6&8 kGy) was examined. Zone diameters due to virulence factors (hemolysin, lecithinase and protease) produced by irradiated spores of both tested strains recorded in table (6) showed correlation between increase in irradiation dose level and decrease in zone diameter. It was observed that dose 8kGy decreased 26, 25&26% of hemolysin, lecithinase& protease enzymes produced by BC13 as well as 16, 17&15% in case of BC37, respectively. In spite of the mentioned correlation, yet the surviving spores were capable of producing their virulence factors confirming the high radiation resistance of *B. cereus* endospores. Previous results reported by Kamat *et al.* (1987) cleared that gamma irradiation had no effect on *B. cereus* lethality. Similarly, Zahran *et al.* (2008) observed that exposure of *B. cereus* spores to 10 kGy gamma irradiation did not affect its toxicity.

Since the stationary phase at which endospores are formed would probably be reached in most abused food, thus inhibiting spore germination and controlling vegetative cells of *B. cereus* has to be the approach in order to prevent and control the spread of this pathogen.

Table 1: Bacterial burden of Corn snacks samples

Samples No.	Viable Count(cfu/gm)				
	Total mesophilic bacteria	Spore forming bacteria			
		Total spore formersat 75°C	*Bacillus cereus* group on MYP agar		
			No. of +ve samples / no. of tested samples	cfu/g	Frequency(%) of +ve samples
1	$6.4x10^4$	1.3x10	0/3	-	0
2	$7.6x10^4$	$2.0x10^2$	2/3	1.5x10	7.5
3	$7.5x10^3$	6.0x10	0/3	-	0
4	$3.6x10^3$	4.4x10	0/3	-	0
5	$5.3x10^6$	$6.6x10^4$	3/3	$5.2x10^3$	7.9
6	$1.8x10^5$	$4.5x10^3$	2/3	$2.0x10^2$	4.4
7	$7.0x10^3$	5.0x10	0/3	-	0
8	$5.5x10^3$	3.6x10	0/3	-	0
9	$3.8x10^5$	$2.1x10^3$	3/3	$8.3x10^2$	39.5
10	$4.5x10^5$	$1.3x10^4$	1/3	$6.4x10^2$	4.9
11	$5.0x10^6$	$2.3x10^4$	3/3	$6.1x10^3$	26.5
12	$8.0x10^5$	$7.2x10^3$	2/3	$2.0x10^2$	2.8
13	$1.7x10^6$	$8.6x10^3$	1/3	$1.6x10^3$	18.6
14	$7.3x10^5$	$3.3x10^2$	1/3	2.0x10	6.1
15	$8.6x10^6$	$2.1x10^4$	2/3	$2.0x10^3$	9.5
16	$3.7x10^5$	$5.8x10^3$	2/3	$1.8x10^2$	3.1
17	$6.2x10^5$	$3.7x10^2$	3/3	4.0x10	10.8
18	$9.8x10^4$	$2.8x10^3$	3/3	$3.8x10^2$	13.6
19	$6.4x10^2$	≤100	0/3	-	0
20	$3.0x10^5$	$3.2x10^2$	3/3	4.0x10	12.5
S.D	1.13	1.13		1.67	10.18
C.D.	21.77	37.92		88.36	1.21
t-value	20.61	11.719		5.08	3.69
Sign.	0.00	0.00		0.00	0.002

NB: cfu/g are mean values of 3 replicates
Correlation is significant at P ≤ 0.01 level .

Table 2: Effect of increasing doses of gamma radiation on viability of *Bacillus spp* contaminating corn snacks samples.

DosekGy	Corn snacks samples									
	No 9		No 11		No 13		No 18		No 20	
	cfu/g	Survival%	cfu/g	Survival%	cfu/g	Survival%	cfu/g	Survival%	cfu/g	Survival%
Cont	$8.3x10^2$	100	$6.1x10^3$	100	$1.6x10^3$	100	$3.8x10^2$	100	$3.3x10^2$	100
1	$8.0x10^2$	96.4	$6x10^3$	98.4	$1.6x10^3$	100	$3.3x10^2$	86.8	$3.5x10^2$	92.1
2	$7.5x10^2$	90.4	$5.4x10^3$	88.5	$1.2x10^3$	75	$2.1x10^2$	55.3	$3x10^2$	78.9
3	$6.5x10^2$	78.3	$4.5x10^3$	73.8	$9.5x10^2$	59.4	$1.8x10^2$	47.4	$2.6x10^2$	68.4
4	$3.4x10^2$	41	$4x10^3$	65.6	$8.8x10^2$	55	$1x10^2$	26.3	$2.2x10^2$	57.9
5	$3.0x10^2$	36.1	$8.5x10^2$	13.9	$7.6x10^2$	47.5	≤10	≤2	$1.7x10^2$	44.7
6	$1.0x10^2$	12	$4.8x10^2$	7.9	$6.6x10^2$	41.25	-	-	$1.1x10^2$	28.9
7	≤100	≤10	$2.2x10^2$	3.6	$2x10^2$	12.5	-	-	≤100	≤10
8	-	-	≤10	≤2	≤100	≤10	-	-	-	-
9	-	-	-	-	-	-	-	-	-	-
10	-	-	-	-	-	-	-	-	-	-
S.D		34.77		41.45		29.76		63.16		25.47
C.D.		53.58		73.41		48.52		47.4		37.86
t-value		4.94		3.85		5.83		4.72		6.99
Sign.		0.003		0.006		0.001		0.009		0.00

NB: cfu/g are mean values of 3 replicates
Correlation is significant at P≤0.01 level.

Table 3: Frequency of *B. cereus* among the isolated cereus group

Sample No	No of isolates	Identification of strains				Frequency of *B. cereus*
		B.cereus	*B.mycoides*	*B.thuringensis*	*B. anthracis*	
9	5	2	3	-	-	40%
11	15	9	6	-	-	60%
13	8	3	3	2	-	37.5%
18	5	3	2	-	-	60%
20	5	4	1	-	-	80%
Total	38	21	15	2	-	55.3%

Table 4: Determination of virulence factors of selected *B. cereus* strains

Sample No	Code No of BC strains	Zone in (mm)		
		Hemolysin	Lecithinase	Protease
9	1	41	43	28
	2	34	22	24
11	6	32	34	30
	7	26	28	27
	8	24	33	33
	9	38	32	31
	10	40	24	25
	11	21	30	35
	12	37	30	22
	13	45	52	38
	14	26	29	33
13	21	38	32	30
	22	25	39	26
	23	19	26	30
18	29	40	34	32
	30	38	27	23
	31	36	33	31
20	34	16	28	34
	35	28	26	27
	36	38	22	21
	37	48	46	45
S.D		8.82	7.7	5.68
C.D.		26.84	24.14	19.09
t-value		17.08	18.99	24.00
Sign.		0.00	0.00	0.00

NB: zone diameters are mean values of 3 replicates
Correlation is significant at $P \leq 0.01$ level.

Table 5: Production of virulence factors by most potent strains of *B. cereus* (BC13 and BC37) after 24 h. and at different incubation temperatures (7, 20, 35, 45, 55°C).

Incubation temp.°C	Zone in mm					
	BC13			BC37		
	Hemolysin	Lecithinase	Protease	Hemolysin	Lecithinase	Protease
7	28	32	24	22	27	29
20	33	46	32	36	44	37
35	45	52	38	48	46	45
45	44	52	37	45	45	42
55	14	20	13	18	10	12
S.D	7.79	8.41	5.54	10.38	7.85	6.14
C.D.	20.08	18.13	16.89	26.75	19.53	16.24
t-value	11.14	12.33	19.24	8.36	11.44	13.77
Sign.	0.00	0.00	0.00	0.00	0.00	0.00

NB zone diameters are mean values of three replicates
Correlation is significant at $P \leq 0.01$ level.

Table 6: Virulence factors produced by irradiated spores of highly potent strains of *B. cereus* (BC13 and BC37)

Dose kGy	Zone in mm					
	BC13			BC37		
	Hemolysin	Lecithinase	Protease	Hemolysin	Lecithinase	Protease
0	45	52	38	48	46	45
1	45	54	38	47	47	48
2	42	47	38	45	46	45
4	40	44	32	45	44	42
6	38	42	28	41	40	38
8	33	39	28	40	38	38
S.D	4.59	5.82	4.97	3.2	3.67	4.08
C.D.	11.33	12.56	14.76	7.22	8.44	9.56
t-value	21.6	19.5	16.6	33.89	29.0	25.6
Sign.	0.00	0.00	0.00	0.00	0.00	0.00

NB: Zones diameters are mean values of three replicates
Correlation is significant at $P \leq 0.01$ level.

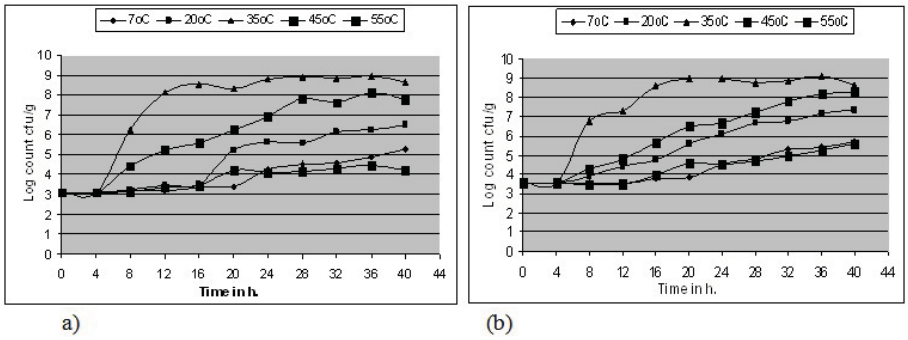

Fig. 1.a.b: Growth of *B. cereus* BC13 and BC37 at different incubation temperatures (7, 20, 35, 45 and 65 °C).

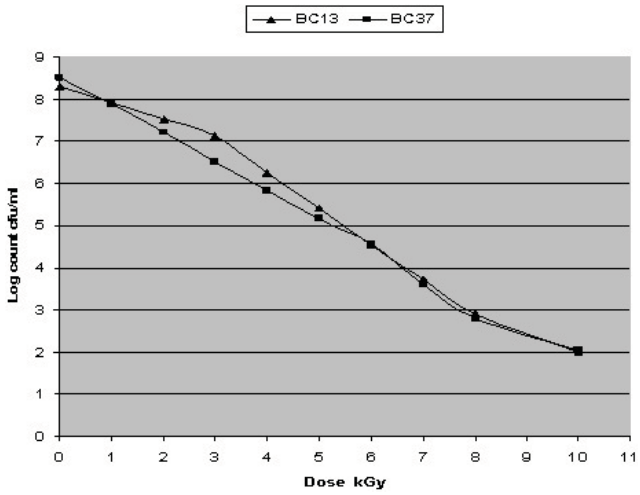

Fig. 2: Survival curves of most potent strains of *B. cereus* (BC13 and BC37) subjected to increasing doses of gamma rays after 24 h. incubation at 35°C D_{10} values of BC13 and BC37 are 2.9 and 2.8 kGy, respectively.

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