

Full Length Research Paper

# Growth of *Bacillus cereus* isolated from some traditional condiments under different regimens

B. M. Okanlawon<sup>1\*</sup>, S. T. Ogunbanwo<sup>2</sup> and A. O. Okunlola<sup>2</sup>

<sup>1</sup>Department of Biomedical Sciences, Ladoké Akintola University of Technology, Ogbomosho, Oyo State, Nigeria.

<sup>2</sup>Department of Botany and Microbiology, University of Ibadan, Nigeria.

Accepted 21 April, 2009

*Bacillus cereus* is ubiquitous in nature and constitutes a major portion of the microbial flora of food contaminating various food samples, causing food spoilage and poisoning to the detriment of the consumers. This work was designed to study the growth characteristics of *B. cereus* strains isolated from traditional condiments under different growth conditions. 34 *Bacillus* strains were isolated from 4 local condiments iru (fermented *Parkia bioglobosa* seeds), ogiri (fermented *Citrullus vulgaris* seeds), dawadawa (fermented soy bean-*Glycine max* seeds) and okpehe (fermented *Prosopis africana* seeds) and identified as *B. cereus*, *B. subtilis*, *B. pumilus* and *B. licheniformis*. *B. cereus* had the highest occurrence of 38.24%. All the *B. cereus* strains had their optimum and minimum growth at 37 and 4°C, respectively, while none grew at 100°C and at pH 1 and 12 when incubated for 48 h. All the *B. cereus* isolates had their highest growth at 10% concentration of monosodium glutamate and the lowest at 40% but their growth pattern in NaCl is strain dependent with optimum growth between 7 and 9% NaCl concentration, and as the time of exposure to ultraviolet light increased the growth decreased.

**Key words:** *Bacillus cereus*, growth kinetics, different regimens, condiments.

## INTRODUCTION

Bacilli, which is widely distributed in nature, constitutes a major portion of the microbial flora of food, easily contaminate various food samples, causing spoilage because of their proteolytic, lipolytic and saccharolytic activities (Kalogridou-vassiliadou, 1992) and food poisoning (Ynte et al., 2004). The genus has been found to be ubiquitous in nature, being found in water bodies, soils and foods. *Bacillus* has also been isolated from extreme environments like hot lakes of temperature greater than 60°C, deep sea (Gaill, 1993; Jannasch and Taylor, 1984), refrigerated foods and high pressure environments (Csonka, 1989). The species in the genus *bacillus* (both free living and pathogenic) include *B. cereus*, *B. anthracis*, *B. thuringensis*, *B. sphaericus*, *B. subtilis*, *B. alcalophilus*, *B. amyloliquefaciens*, *B. fastidious*, *B. infernos*, *B. licheniformis*, *B. megaterium*, *B. polymyxa*, *B. popilliae*, *B. psychrophilus*, *B. pasteurii* and *B. stearothermophilus* (Prescott et al., 2005).

Two *bacillus* species are considered medically signifi-

cant, *B. anthracis* which causes anthrax and *B. cereus* which causes a food borne illness similar to that of *Staphylococcus*. Other species such as *B. thuringensis* and *B. sphaericus* are important insect pathogens and are sometimes used to control insect pests (Lambert and Peferoen, 1992). The species *B. subtilis* is an important model organism, a notable food spoiler causing ropiness in bread and related foods (Wikipedia, 2008). Many species of *Bacillus* are of considerable importance in the production of antibiotics such as bacitracin, gramicidin and polymyxin (Prescott et al., 2005).

The presence of psychrotrophic enterotoxigenic *B. cereus* in ready to serve meats and meat products that have not been subjected to sterilization treatment is a public health concern. *B. cereus* causes problems to the food industry both by deteriorating the products (Te Giffel et al., 1996) and by endangering people's life upon consuming them (Granum et al., 1993, Ghelardi et al., 2002). Under certain conditions, strains of this species produce haemolysis, phospholipases C and enterotoxin that cause food poisoning (Rusul and Yaacob, 1995). There have been an increasing number of food poison outbreaks caused by *B. cereus* (Gilbert et al., 1981; Johnson,

\*Corresponding author. E-mail: okantunde@yahoo.com.

1984). Dairy products such as raw and pasteurized milk, cheese and ice cream have been reported to be frequently contaminated with *B. cereus* (Ahmed et al., 1983; Wong et al., 1988). The factors that make *B. cereus* a potential threat to food processing are ability to form thermotolerant endospore, ability to grow and survive at refrigeration temperature and toxin production (Van Netten et al., 1990; Griffiths, 1990; Grannum and Lund, 1997; O'Mahony et al., 2001).

Production of  $\beta$ -lactamases is one of the potential virulence factors that make the strains resistant even to the 3<sup>rd</sup> generation of cephalosporins (Cormican et al., 1998). The purpose of this work is to examine the effect of different growth parameters on the survival and proliferation of *B. cereus* in order to have the technical-know-how of controlling their growth during food processing.

## MATERIALS AND METHODS

### Sample collection

Four different local condiments iru, dawadawa, ogiri and okpeke were bought from Bodija market, a central market in Ibadan, Oyo State, Nigeria. The samples were collected in sterile white polythene containers and transferred to the laboratory for microbial analysis.

### Isolation and characterization of *Bacillus* species

*Bacillus* species were isolated using the method of Olutiola et al. (1991). Characterization and Identification of the isolates were done using the conventional colonial morphology, Gram staining and biochemical reactions according to Bergey's manual of systems bacteriology (Sneath, 1986).

### Effect of temperature on growth of *Bacillus cereus*

5 ml sterile nutrient broth in test tubes were inoculated with the test organisms in 3 replicates and incubated at different temperatures (4, 30, 37, 55 and 65°C) for 24 h. After incubation, growth was measured using the uniscope 23D (Uniscope England) spectrophotometer at 540 nm.

### Effect of pH on growth of *B. cereus*

5 ml of nutrient broth at different pH (3, 5, 8, 9 and 10) was inoculated with the test organisms in 3 replicates and incubated at 30°C for 24 h after which growth was measured using the uniscope 23D spectrophotometer at 540 nm.

### Effect of incubation period on growth of *B. cereus*

Inoculated broth cultures of the test organisms were incubated at 30°C for different times in 3 replicates after which growths were measured with spectrophotometer at 540 nm.

### Effect of monosodium glutamate concentration on growth of *B. cereus*

1, 10, 20 and 40% concentrations of monosodium glutamate were

prepared using nutrient broth as diluent. These were sterilized and inoculated with test organisms in 3 replicates and incubated 30°C for 24 h. Growths were measured using Unispec 23D spectrophotometer at 540 nm.

### Effect of different NaCl concentrations on the growth of *B. cereus* in meat extract broth

Meat extract broth was prepared as described by Olutiola (1991), inoculated in 3 replicates and incubated at 30°C for 24 h after which growths were measured as described above.

### Effect of different NaCl concentrations on the growth of *B. cereus* in locust beans

Locust beans were dried in the oven and ground to powder. 10 g of the powder was added to 1 l of distilled water to make 1% solution. 15 g of glucose was also added and dispensed 5 ml of the aliquot in test tubes. The broth was sterilized in the autoclave at 121°C for 15 min and allowed to cool down. The broth cultures were inoculated with the test organisms in 3 replicates and incubated at 30°C for 24 h after which growths were measured.

### Effect of ultraviolet light on the growth of *B. cereus*

5 ml of sterile nutrient broth was inoculated with the test organisms. The broth cultures of the organisms were placed in sterile petri-dishes and were exposed to ultraviolet light from a 15 W general electronic germicidal bulb at a distance of 15 cm for 0, 2, 4, 6, 18, and 24 h in 3 replicates. After exposure to ultraviolet light, growths were measured using unispec 23D spectrophotometer at 540 nm.

### Statistical analysis

Data were subjected to analysis of variance (ANOVA) means separated by Duncan's multiple range test.

## RESULTS

34 bacteria strains were isolated from 4 local condiments iru, ogiri, dawadawa and okpeke. The isolates were identified based on their morphological appearance, physiological and biochemical tests which include Gram-staining, spore staining, catalase and indole test, growth at 7% NaCl concentration, gelatin liquefaction, starch hydrolysis, nitrogen reduction and sugar fermentation test. The isolates were identified as *B. cereus*, *B. subtilis*, *B. pumilus* and *B. licheniformis*. All the isolates were Gram-positive endospore forming rods, catalase positive, liquefied gelatin and indole negative. Only *B. cereus* grew in 7% NaCl concentration and most hydrolysed starch. Majority of the isolates fermented glucose, inulin and fructose while most of the isolates fermented mannitol. All the isolates that were later identified as *B. cereus* did not ferment mannitol and arabinose (Table 1)

The frequency and occurrence of *Bacillus* species isolated from condiments are shown in Table 2. *Bacillus cereus* had the highest occurrence of 38.24% while *B.*

**Table 1.** Characteristics of *Bacillus* species isolated from Nigerian condiments

Test	Species I	Species II	Species III	Species IV
Gram's reaction	+	+	+	+
Catalase	+	+	+	+
Spores	+	+	+	+
Starch hydrolysis	+	+	-	+
Growth in 7% NaCl	+	-	-	-
Nitrate reduction	+	+	-	+
Gelatin liquefaction	+	+	+	+
Indole production	-	-	-	-
Methyl red test	-	-	-	+
Voge's Proskaver test	+	+	-	+
Citrate Utilization	+	+	+	+
Urease activity	-	-	-	-
Mannitol	-	+	+	+
Lactose	+	+	-	-
Arabinose	-	+	+	+
Xylose	-	+	+	+
Glucose	+	+	+	+
Galactose	+	-	+	+
Maltose	+	-	+	+
Inulin	+	+	-	+
Raffinose	+	-		-
Sucrose	+	+	+	+
Fructose	+	+	+	+
Anaerobic growth	+	-	-	+
Hemolysis of RBC	+	+	+	+
Probable identity	<i>B. cereus</i>	<i>B. subtilis</i>	<i>B. pumilus</i>	<i>B. licheniformis</i>

*pumilus* had the lowest (11.76%). However, *B. subtilis* and *B. licheniformis* had occurrences of 35.30% and 14.70%, respectively.

All the *B. cereus* isolated in this work had their optimum and minimum growth at 37 and 4°C respectively while none grew at 100°C. *B. cereus* Og004 recorded the highest growth with optical density (O.D.) of  $1.628 \pm 0.16$  at 37°C while *B. cereus* Ir003 had the least growth at 37°C with O.D. of  $1.316 \pm 0.23$  (Table 3)

Table 4 shows the effect of pH on the growth of the *B. cereus* isolates. *B. cereus* Ir003 recorded its highest growth with OD of  $1.622 \pm 0.03$  at pH 8, *B. cereus* Ir009, *B. cereus* Og004 and *B. cereus* Nu003 recorded their highest growth with OD of  $1.622 \pm 0.03$ ,  $1.592 \pm 0.17$  and  $1.296 \pm 0.15$  respectively at pH 9, while *B. cereus* Yo 002 had its highest growth with OD of  $1.516 \pm 0.12$  at pH 10.

On the effect of different incubation periods, *B. cereus* Og004 recorded the highest growth with OD of  $1.732 \pm 0.11$  at 48 h while *B. cereus* Nu003 recorded the lowest growth with OD of  $1.021 \pm 0.01$  at 120 h (Table 5).

Studying the effect of monosodium glutamate at different concentrations of 1, 10, 20, and 40% on the growth of *B. cereus*, it was generally observed that the cell morphology was affected, the rod shape was drastically

reduced to dots, almost coccal in shape (data not shown). All the test isolates had their highest growth at 10% concentration of monosodium glutamate with *B. cereus* Ir009 having the highest growth of  $1.355 \pm 0.01$  O.D and *B. cereus* Ir003 had the lowest growth with OD of  $1.072 \pm 0.06$  at 10% concentration. All the test isolates had their lowest growth at 40% concentration of monosodium glutamate with *B. cereus* Ir009 having the lowest OD value of  $0.515 \pm 0.04$  (Table 6).

The effect of NaCl concentration on the growth of *B. cereus* in medium containing 1% glucose as the carbon source and 1% locust bean as the nitrogen source is shown in Table 7. *B. cereus* Yo 002 had the highest growth with OD of  $1.192 \pm 0.30$  at 9% NaCl concentration. However, when locust bean was substituted with meat extract as nitrogen source, *B. cereus* Og004 recorded the highest growth with OD of  $1.234 \pm 0.03$  at 9% NaCl concentration although the growth pattern is strain specific (Table 8).

Table 9 shows the effect of ultraviolet light on the growth of *B. cereus* isolates. As the time of exposure to ultraviolet light increased the growth decreased with *B. cereus* Og004 having the highest growth with OD of  $1.227 \pm 0.02$  at 0 h and decreased to  $0.582 \pm 0.10$  at the

**Table 2.** % occurrence of *Bacillus* species isolated from Nigerian condiments.

Isolates	Condiments	Number	Percentage (%)
<i>B. cereus</i>	Iru	6	17.65
	Ogiri	5	14.71
	Dawadawa	2	5.88
	Okpehe	-	-
<b>Total</b>		<b>13</b>	<b>38.24</b>
<i>B. subtilis</i>	Iru	3	8.823
	Ogiri	2	5.88
	Dawadawa	4	11.77
	Okpehe	3	8.823
<b>Total</b>		<b>12</b>	<b>35.30</b>
<i>B. pumilus</i>	Iru	2	5.88
	Ogiri	-	-
	Dawadawa	2	5.88
	Okpehe	-	-
<b>Total</b>		<b>4</b>	<b>11.76</b>
<i>B. licheniformis</i>	Iru	1	2.94
	Ogiri	-	-
	Dawadawa	3	8.823
	Okpehe	1	2.94
<b>Total</b>		<b>5</b>	<b>4.70</b>
Grand total		34	100

**Table 3.** Effect of temperature on growth of *Bacillus cereus* strains isolated from Nigerian condiments.

Isolate	Temperature					
	4 °C	30 °C	37 °C	55 °C	65 °C	100 °C
<i>B. cereus</i> Ir003	0,628 ± 0.12 <sub>a</sub>	1.106 ± 0.14 <sub>b</sub>	1.316 ± 0.23 <sub>c</sub>	1.279 ± 0.13 <sub>d</sub>	0.828 ± 0.32 <sub>e</sub>	0.00
<i>B. cereus</i> Ir009	0.778 ± 0.31 <sub>ab</sub>	1.413 ± 0.40 <sub>bb</sub>	1.554 ± 0.14 <sub>ca</sub>	1.318 ± 0.23 <sub>da</sub>	0.944 ± 0.12 <sub>ea</sub>	0.00
<i>B. cereus</i> Og004	0.825 ± 0.34 <sub>ac</sub>	1.482 ± 0.25 <sub>bc</sub>	1.628 ± 0.16 <sub>cb</sub>	1.461 ± 0.43 <sub>db</sub>	1.002 ± 0.04 <sub>eb</sub>	0.00
<i>B. cereus</i> Yo002	0.659 ± 0.20 <sub>ad</sub>	1.155 ± 0.15 <sub>bd</sub>	1.321 ± 0.31 <sub>cc</sub>	1.136 ± 0.25 <sub>dc</sub>	0.865 ± 0.35 <sub>ec</sub>	0.00
<i>B. cereus</i> Nu003	0.711 ± 0.16 <sub>ae</sub>	1.114 ± 0.35 <sub>be</sub>	1.333 ± 0.30 <sub>cd</sub>	1.162 ± 0.13 <sub>dd</sub>	0.816 ± 0.10 <sub>ed</sub>	0.00

end of 24 h exposure.

## DISCUSSION

This study was conducted in Ibadan, South west Nigeria, where many tribes with different cultural backgrounds reside that use different condiments due to their cultural backgrounds and life styles.

*Bacillus* species were isolated from some Nigerian traditional condiments iru, ogiri, dawadawa and okpehe. This agrees with the work of Marrku and Constantin (1975), Ternstrom et al. (1993), Schraft et al. (1996) and O' Mahony et al. (2001), who have equally isolated *Bacillus* species from these sources. The cultural and biochemical characteristics of the *Bacillus* sp. isolated in

this work were confirmed with the Bergey's manual of systematic bacteriology (Sneath et al., 1986). The *Bacillus* species isolated in this work are *B. subtilis*, *B. pumilus*, *B. licheniformis* and *B. cereus*. The use of 7% NaCl concentration in media to screen out *B. cereus* from all the *Bacillus* species was adopted in consonance with Olutiola et al. (1991).

The occurrence of *B. cereus* in higher proportion than other species in most of the condiments may be due to the fact that *B. cereus* is more adaptable to a wider varying environment than other species.

The effect of temperature on the growth of *B. cereus* showed that all the *B. cereus* isolates grew best at 37°C while the least growth was observed at 4°C. However, none of the isolates grew at 100°C thus confirming the work of Johnson and Snygg (1974) who reported that the

**Table 4.** Effect of pH on growth of *B. cereus* strains isolated from Nigerian condiments

Isolate	pH						
	1	3	5	8	9	10	12
<i>B. cereus</i> Ir003	0.00	1.47 ± 0.01 <sub>a</sub>	1.616 ± 0.04 <sub>b</sub>	1.771 ± 0.05 <sub>c</sub>	1.444 ± 0.12 <sub>d</sub>	1.452 ± 0.02 <sub>e</sub>	0.00
<i>B. cereus</i> Ir009	0.00	1.161 ± 0.09 <sub>b</sub>	1.373 ± 0.02 <sub>ba</sub>	1.613 ± 0.13 <sub>ca</sub>	1.622 ± 0.03 <sub>da</sub>	1.564 ± 0.14 <sub>ea</sub>	0.00
<i>B. cereus</i> Og004	0.00	1.568 ± 0.02 <sub>c</sub>	1.571 ± 0.06 <sub>bc</sub>	1.571 ± 0.05 <sub>cb</sub>	1.592 ± 0.17 <sub>db</sub>	1.537 ± 0.08 <sub>eb</sub>	0.00
<i>B. cereus</i> Yo002	0.00	1.257 ± 0.0 <sub>d</sub>	1.604 ± 0.32 <sub>bd</sub>	1.303 ± 0.16 <sub>cc</sub>	1.472 ± 0.10 <sub>dc</sub>	1.516 ± 0.12 <sub>ec</sub>	0.00
<i>B. cereus</i> Nu003	0.00	0.932 ± 0.04 <sub>e</sub>	1.176 ± 0.01 <sub>be</sub>	1.284 ± 0.10 <sub>cd</sub>	1.296 ± 0.15 <sub>dd</sub>	1.147 ± 0.05 <sub>ed</sub>	0.00

**Table 5.** Effect of incubation time on growth of *B. cereus* strains isolated from Nigerian condiments.

Isolate	Incubation period				
	24 h	48 h	72 h	96 h	120 h
<i>B. cereus</i> Ir003	1.422 ± 0.02 <sub>a</sub>	1.436 ± 0.31 <sub>b</sub>	1.221 ± 0.04 <sub>c</sub>	1.501 ± 0.10 <sub>d</sub>	1.176 ± 0.05 <sub>e</sub>
<i>B. cereus</i> Ir009	1.022 ± 0.16 <sub>ab</sub>	1.227 ± 0.07 <sub>bb</sub>	1.525 ± 0.18 <sub>ca</sub>	1.711 ± 0.08 <sub>da</sub>	1.517 ± 0.12 <sub>ea</sub>
<i>B. cereus</i> Og004	1.624 ± 0.20 <sub>ac</sub>	1.732 ± 0.11 <sub>bc</sub>	1.604 ± 0.02 <sub>cb</sub>	1.614 ± 0.17 <sub>db</sub>	1.695 ± 0.20 <sub>eb</sub>
<i>B. cereus</i> Yo002	1.089 ± 0.13 <sub>ad</sub>	1.282 ± .05 <sub>bd</sub>	1.252 ± 0.14 <sub>cc</sub>	1.268 ± 0.11 <sub>dc</sub>	1.208 ± 0.16 <sub>ec</sub>
<i>B. cereus</i> Nu003	1.396 ± 0.20 <sub>ae</sub>	1.396 ± 0.12 <sub>be</sub>	0.885 ± 0.10 <sub>cd</sub>	1.048 ± 0.19 <sub>dd</sub>	1.021 ± 0.01 <sub>ed</sub>

**Table 6.** Effect of monosodium glutamate concentration on growth of *B. cereus* strains isolated from Nigerian condiments.

Isolate	Monosodium Glutamate			
	1%	10%	20%	40%
<i>B. cereus</i> Ir003	0.923 ± 0.07 <sub>a</sub>	1.072 ± 0.06 <sub>b</sub>	0.632 ± 0.09 <sub>c</sub>	0.558 ± 0.01 <sub>e</sub>
<i>B. cereus</i> Ir009	1.142 ± 0.05 <sub>ab</sub>	1.355 ± 0.01 <sub>bb</sub>	0.572 ± 0.03 <sub>ca</sub>	0.515 ± 0.04 <sub>ea</sub>
<i>B. cereus</i> Og004	0.978 ± 0.01 <sub>ac</sub>	1.174 ± 0.06 <sub>bc</sub>	0.686 ± 0.08 <sub>cb</sub>	0.524 ± 0.02 <sub>eb</sub>
<i>B. cereus</i> Yo002	0.924 ± 0.04 <sub>a</sub>	1.155 ± 0.03 <sub>bd</sub>	0.587 ± 0.01 <sub>cc</sub>	0.522 ± 0.07 <sub>eb</sub>
<i>B. cereus</i> Nu003	0.978 ± 0.02 <sub>ac</sub>	1.161 ± 0.08 <sub>be</sub>	0.569 ± 0.05 <sub>ca</sub>	0.526 ± 0.03 <sub>eb</sub>

**Table 7.** Effect of NaCl concentration on growth of *B. cereus* strains in locust bean broth.

Isolate	NaCl concentration			
	4%	7%	9%	10%
<i>B. cereus</i> Ir003	1.046 ± 0.01 <sub>a</sub>	1.094 ± 0.03 <sub>b</sub>	1.078 ± 0.06 <sub>c</sub>	0.906 ± 0.01 <sub>d</sub>
<i>B. cereus</i> Ir009	1.094 ± 0.04 <sub>ab</sub>	1.174 ± 0.04 <sub>bb</sub>	1.054 ± 0.01 <sub>ca</sub>	1.032 ± 0.03 <sub>da</sub>
<i>B. cereus</i> Og004	1.071 ± 0.08 <sub>ac</sub>	1.132 ± 0.02 <sub>bc</sub>	1.152 ± 0.01 <sub>cb</sub>	0.055 ± 0.02 <sub>db</sub>
<i>B. cereus</i> Yo002	1.088 ± 0.03 <sub>ad</sub>	1.094 ± 0.02 <sub>b</sub>	1.192 ± 0.30 <sub>cc</sub>	0.949 ± 0.06 <sub>dc</sub>
<i>B. cereus</i> Nu003	1.078 ± 0.03 <sub>ae</sub>	1.102 ± 0.06 <sub>be</sub>	1.089 ± 0.12 <sub>cd</sub>	0.924 ± 0.04 <sub>dd</sub>

optimum temperature for *B. cereus* is between 30 and 37°C but some strains can grow at temperature as low as 4.5°C and up to 55°C.

The influence of pH on the growth of *B. cereus* revealed that most of the strains tested had their optimum growth at pH 9. However, pH 1 and 12 were found to be inhibitory to growth of *B. cereus*. Its significance to food protection is minimized by the fact that very few foods have such a high pH. This finding is in agreement with

the work of Goepfert et al. (1972) who reported that pH 4.9 - 9.3 permitted growth of *B. cereus* in laboratory media.

According to Prescott et al. (2005) and Ynte et al. (2004), *B. cereus* is able to grow between 18 to 48 h in laboratory media. In this study, most of the tested isolates had their highest growth at 48 h while at 72 h the growth declined and later increased at 96 h. This may be due to the fact that after 48 h most vegetative cells have sporu-

**Table 8.** Effects of NaCl concentration on growth of *B. cereus* strains in meat extract broth.

Isolate	NaCl concentration			
	4%	7%	9%	10%
<i>B. cereus</i> Ir003	0.778 ± 0.10 <sub>a</sub>	0.821 ± 0.02 <sub>b</sub>	0.918 ± 0.01 <sub>c</sub>	0.703 ± 0.03 <sub>d</sub>
<i>B. cereus</i> Ir009	0.720 ± 0.07 <sub>ab</sub>	0.794 ± 0.04 <sub>ba</sub>	0.723 ± 0.05 <sub>ca</sub>	0.713 ± 0.08 <sub>da</sub>
<i>B. cereus</i> Og004	0.813 ± 0.04 <sub>ac</sub>	0.926 ± 0.01 <sub>bb</sub>	1.234 ± 0.03 <sub>cb</sub>	0.818 ± 0.10 <sub>db</sub>
<i>B. cereus</i> Yo002	0.753 ± 0.02 <sub>ad</sub>	0.816 ± 0.03 <sub>bc</sub>	0.918 ± 0.04 <sub>c</sub>	0.744 ± 0.06 <sub>dc</sub>
<i>B. cereus</i> Nu003	0.746 ± 0.01 <sub>ae</sub>	0.788 ± 0.03 <sub>bd</sub>	0.717 ± 0.01 <sub>cc</sub>	0.639 ± 0.02 <sub>dd</sub>

**Table 9.** Effect of ultraviolet light on growth of *B. cereus* strains isolated from Nigerian condiments.

Isolate	Period (h)					
	0	2	4	6	18	24
<i>B. cereus</i> Ir003	0.756 ± 0.08 <sub>a</sub>	0.672 ± 0.10 <sub>b</sub>	0.560 ± 0.02 <sub>c</sub>	0.512 ± 0.05 <sub>d</sub>	0.504 ± 0.01 <sub>e</sub>	0.462 ± 0.01 <sub>f</sub>
<i>B. cereus</i> Ir009	0.773 ± 0.10 <sub>ab</sub>	0.713 ± 0.08 <sub>ba</sub>	0.614 ± 0.05 <sub>ca</sub>	0.564 ± 0.0 <sub>da</sub>	0.531 ± 0.04 <sub>ea</sub>	0.517 ± 0.06 <sub>fa</sub>
<i>B. cereus</i> Og004	1.227 ± 0.02 <sub>ac</sub>	0.784 ± 0.10 <sub>bc</sub>	0.702 ± 0.03 <sub>cb</sub>	0.642 ± 0.01 <sub>db</sub>	0.612 ± 0.01 <sub>eb</sub>	0.582 ± 0.10 <sub>fb</sub>
<i>B. cereus</i> Yo002	0.768 ± 0.07 <sub>ad</sub>	0.685 ± 0.04 <sub>bc</sub>	0.564 ± 0.01 <sub>c</sub>	0.532 ± 0.07 <sub>dc</sub>	0.512 ± 0.04 <sub>ec</sub>	0.501 ± 0.03 <sub>fc</sub>
<i>B. cereus</i> Nu003	0.757 ± 0.10 <sub>a</sub>	0.644 ± 0.12 <sub>bd</sub>	0.573 ± 0.09 <sub>cd</sub>	0.551 ± 0.04 <sub>dd</sub>	0.521 ± 0.01 <sub>ed</sub>	0.506 ± 0.05 <sub>fd</sub>

lated and more of spores remained in the culture than vegetative cells. After 72 h the spores started germinating again which led to increase in vegetative cells at 96 h.

The *B. cereus* strains, isolated in this study had their highest growth in medium containing 10% monosodium glutamate concentration and the lowest at 40% monosodium glutamate concentration when monosodium glutamate was used as the source of carbon. This shows that higher concentrations (above 10%) of monosodium glutamate retarded growth of *B. cereus* confirming the work of Ynte et al. (2004) which indicates that *B. cereus* can grow and sporulate on chemically defined medium without glucose as the carbon source.

Growth at different concentrations of NaCl in locust bean and meat extract broth as nitrogen sources shows that as the NaCl concentration increases, growth decreases but with variation among strains. This confirms the earlier work of Troller (1973). Most of the tested isolates recorded their highest growth at 8% NaCl concentration in medium containing locust bean as nitrogen source while the rest recorded their highest growth at 9% NaCl concentration in medium containing meat extract as nitrogen source. This agrees with Marrku and Constantin (1975) who reported that the effect of NaCl on growth of *B. cereus* varies with strains and growth medium used.

When exposed to ultraviolet light, as the number of hours of exposure increased, growth decreased, since light wavelength between 2400 - 3000 Å (UV spectrum) is lethal to most microorganisms; hence most of the exposed bacteria are killed at this radiation (Dubey and Raheshwar, 2002).

Since *B. cereus* causes food poisoning and spoilage; it is a major threat in food industry both by deteriorating the products (Te Giffed et al., 1996) and by endangering

people's life upon consuming them (Granum et al., 1993; Ghelardi et al., 2002). The findings of this study will help in controlling the incidence and survival of *B. cereus* in raw and ready to serve foods by employing the germicidal effects of monosodium glutamate, sodium chloride and ultraviolet radiation to prevent the growth of *B. cereus* in condiments.

## REFERENCES

- Ahmed AA, Moustafa MK, Marth EH (1983). Incidence of *B. cereus* in milk and some milk products. *J. Food Prot.* 46: 126-128.
- Cormican M, Moris D, Corrbet-Feenney G (1998). Extended spectrum  $\beta$ -lactamase production and fluoroquinolone resistance associated with community acquired urinary tract infection. *Diagn. Microbiol. Infect. Dis.* 32: 377-379.
- Csonka LN (1989). Physiological and genetic responses of bacteria to osmotic pressure. *Microbiol. Rev.* 53(1): 121-147.
- Dubey RC, Raheshwar DK (2002). *Practical Microbiology*. Schand and company Ltd., New Delhi.
- Gaill F (1993). Aspects of life development at the deep sea hydrothermal vents. *FAESB J.* 7: 558-565.
- Goepfert IM, Spira WM, Kim HU (1972). *Bacillus cereus* food poisoning organisms. A review. *J. Milk Food Technol.* p. 213.
- Ghelardi E, Celandroni F, Salvetti S, Barsoti C, Baggiani A, Senesi S (2002). Identification and characterization of toxigenic *Bacillus cereus* isolates responsible for two food-poisoning outbreaks. *FEMS Microbiol. Lett.* 208: 129-134.
- Gilbert RJ, Turnbull PCB, Parry JM, Kramer JM (1981). *B. cereus* and other *Bacillus* species: their part in food poisoning and other clinical infections. pp. 297-314. In R. C. W. Berkely and M. Goodfellow (ed.), *The aerobic endospore-forming bacteria: classification and identification*. Academic press inc., London.
- Granum E, Lund T (1997). *Bacillus cereus* and its food poisoning toxins. *FEMS. Microbiol. Lett.* 157: 223-228.
- Granum PE, Brynestad S, Kramer JM (1993). Analysis of enterotoxin production by *Bacillus cereus* from dairy products, food poisoning incidents and nongastrointestinal infection. *Int. J. Food Microbiol.*, 17: 269-279.

- Griffiths MN (1990). Toxin production by *Bacillus* spp. Present in milk. *J. Food Prot.* 53: 790-792.
- Jannasch HW, Taylor CD (1984). Deep sea Microbiology. *Ann. Rev. Microbiol.* 38: 487-514.
- Johnson KM (1984). *Bacillus cereus* food borne illness- an update. *J. Food prot.* 47: 147-153.
- Johnson U, Snygg BG (1974). Lipase production and activity as a function of incubation, time, pH and temperature of four lipolytic organisms. *J. Appl. Bacteriol.* 37: 571-581.
- Kalogridou-Vassiliadou K (1992). Biochemical activities of *Bacillus* species isolated from flat sour evaporated milk. *J. Dairy Sci.* 75: 2681-2686.
- Lambert B, Peferoen M (1992). Insecticidal promise of *Bacillus thuringiensis*. Facts and mysteries about a successful biopesticides. *Bioscience*, 42(2): 112-122.
- Marrku R, Constantin G (1975). Effect of pH and sodium chloride on growth of *Bacillus cereus* in laboratory media and certain foods. *Appl. Microbiol.* 29(1): 68-73.
- O'mahony T, Rechif N, Cavadini C, Fitzgerald GF (2001). The application of a fermented food ingredients containing Variacin, a novel antimicrobial produced by *Kocuria varians* to control the growth of *Bacillus cereus* in chilled dairy products. *J. Appl. Microbiol.* 85: 17-24.
- Olutiola PO, Famurewa O, Sonntang HG (1991). An introduction to general microbiology; a practical approach. Hygiene institute der Universtat, Heidelberg.
- Prescott LM, Harley PJ, Klein AD (2005). *Microbiol.* 6<sup>th</sup> Ed. McGraw-Hill Companies, Inc., New York, USA, p. 951.
- Rusul G, Yaacob NH (1995). Prevalence of *Bacillus cereus* in selected foods and detection of enterotoxin using TECRA-VIA and BCET-RPLA. *Int. J. Food Microbiol.*, 25: 131-139.
- Schraft H, Steel M, McNab B, Odumeru J, Griffiths MW (1996). Epidemiological typing of *Bacillus* spp. isolated from food. *Appl. Environ. Microbiol.* 62(11): 4229-4232.
- Sneath PA, Mair NS, Sharpe ME, Holts JG (1986). *Bergey's Manual Systematic Bacteriology*, Vol. 2, Baltimore MD: Willam and Wilkins.
- Te Giffel MC, Beumer MC, Slaghuis RR, Rombouts FM (1996). Occurrence and characterization of (psychrotrophic) *Bacillus cereus* on farms in the Netherlands. *Neth. Milk Dairy J.* 49: 125-238.
- Ternstrom A, Lindberg AM, Mohn G (1993). Classification of the spoilage flora of raw and pasteurized bovine milk with special reverence to *Pseudomonas* and *Bacillus*. *J. Appl. Bacteriol.* 75: 25-34.
- Troller JA (1973). The water relation of food borne bacterial pathogen. A review. *J. Milk Food Technol.* 36: 276-288.
- Van Netten P, Van de Moosdijk A, Van Hoensel P, Mossel DA, Perales I (1990). Psychrotrophic strains of *Bacillus cereus* producing enterotoxin. *J. Appl. Bacteriol.*, 69: 73-79.
- Wikipedia (2008). *Bacillus*, The Free Encyclopaedia from Wikipedia founder Jimmy Wales, <http://en.wikipedia.org/wiki/Bacillus>.
- Wong HC, Chang MH, Fan JY (1988). Incidence and characterization of *Bacillus cereus* isolates contaminating dairy products. *Appl. Environ. Microbiol.* 54: 699-702.
- Ynte PDE, Vries Luc M, Hornstra Willem M, Vos Tjakko Abee DE (2004). Growth and Sporulation of *Bacillus cereus* ATCC 14579 under Defined Conditions: Temporal Expression of Genes for Key Sigma Factors. *Appl. Environ. Microbiol.* 70(4): 2514-2519.