IPS Journal of Basic and Clinical Medicine
IPS J Basic & Clin Med, 2(3): 108-114 (2025)
DOI: https://doi.org/10.54117/ijbcm.v2i3.18

Prevalence of *Bacillus cereus* in Powdered Soybean Sold in Uli Community, Anambra State: A Cross-Sectional Study

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Abstract

This study investigated the prevalence of *Bacillus cereus* in powdered soybean samples sold in Uli community, Anambra State. A total 100 of samples were randomly collected from various stalls and shops across different locations in the community. Standard microbiological techniques were employed to isolate and identify *Bacillus* cereus. The results revealed a significant ($p \leq 0.05$) presence of *B. cereus*, with 33.00% of the samples testing positive. Two distinct strains of *B. cereus* were identified: *Bacillus cereus* strain FORC60 (BCFOR) and *Bacillus cereus* strain DQ01 (BCDQO). Statistical analysis showed a significant difference in the prevalence of these strains, with BCFOR being the most predominant (69.70%, $p < 0.05$) compared to BCDQO (30.30%). These findings suggest that *B. cereus* is a common contaminant in powdered soybean products sold in Uli community, posing potential health risks to consumers. The high prevalence of *B. cereus* in powdered soybean products highlights the need for improved food safety measures, including proper handling, storage, and processing practices. *B. cereus* is a known cause of food poisoning, and its presence in food products can lead to gastrointestinal illness. Therefore, regular monitoring and control of *B. cereus* in food products are crucial to preventing foodborne illnesses.

Keywords: Strains, Microbiological, Predominant, Foodborne

Article History

Received: 08 Jun 2025
Accepted: 26 Jun 2025
Published: 28 Jun 2025

Scan QR Code to view[1]

How to cite this paper: Ike, V. E., Iheukwumere, I. H., Iheukwumere, C. M., Dim, C. N., Ezendianefo, J. N., Egbe, P. A., Oragwu, I. P., Orji, C. C., Ogbonnaya, O. C., Onwuasoanya, U. F., Okereke, F. O., & Ochibulu, S. C. (2025). Prevalence of Bacillus cereus in Powdered Soybean Sold in Uli Community, Anambra State: A Cross-Sectional Study. *IPS Journal of Basic and Clinical Medicine, 2*(3), 108–114. https://doi.org/10.54117/ijbcm.v2i3.18

1. Introduction

Bacillus is a Gram-positive, facultative anaerobic and spore-forming rod. *Bacillus* genus includes both food borne pathogens and food spoilage-associated bacteria, such as *B. cereus, B. subtilis, B. licheniformis, B. pumilus, B. weihenstephanensis and B. sporothermodurans* (Gopal *et al*., 2015). *B. cereus* is most commonly detected food pathogen from this genus (Logan, 2011; Tewari and Abdullah, 2015; Gopal *et al*., 2015). Among other members, *B. licheniformis* can cause an enteric disease and food poisoning in humans. Similarly, strains of *B. subtilis* may occasionally cause food poisoning outbreaks involving foods such as milk powder (Fernandez-No *et al*., 2011; Gopal *et al*., 2015). *Bacillus* spp. are widely distributed in the environment with soil as the natural habitat (Tewari and Abdullah, 2015).

Bacillus spores are devoid of metabolic activity and are refractory to extreme environmental conditions such as heat, freezing, drying and radiation. These spores can be transmitted through processed, pasteurized and heat-treated food products. Most strains of bacillus are mesophilic,

having an optimal temperature between 25 ℃ and 37 ℃, and neutralophilic, preferring neutral pH, but some have been found to grow in environments with much more extreme conditions. *B. cereus* has been isolated from a variety of foods, particularly Ready–To–Eat foods such as cooked rice and mixed salad. *B. cereus* can cause food poisoning even at very low doses, with more than 10^3*B. cereus* g^{-1} considered unsafe for consumption. Two types of illness have been attributed to the consumption of foods contaminated with *B. cereus*. The first and better known is characterized by abdominal pain and non-bloody diarrhoea; it has an incubation period of 4-16hours following ingestion with symptoms that last for 12-24 hours. The second, which is characterized by an acute attack of nausea and vomiting, occurs within 1-5hours after consumption of contaminated food; diarrhoea is not a common feature in this type of illness (Riset, 2022).

While *B. cereus* vegetative cells are killed during normal cooking, spores are more resistant. Viable spores in food can become vegetative cells in the intestines and produce a range of diarrheal enterotoxins, so elimination of spores is desirable. In wet heat (poaching, simmering, boiling, braising, stewing, pot roasting, steaming), spores require more than 5 minutes at 121 ℃ (250 °F) at the coldest spot to be destroyed. In dry heat (grilling, broiling, baking, roasting, searing, sautéing), 120 ℃ (248 °F) for 1 hour kills all spores on the exposed surface. This process of eliminating spores is very important, as spores of *B. cereus* are particularly resistant, even after pasteurization or exposure to gamma ray. *B. cereus* and other members of *Bacillus* are not easily killed by alcohol; they have been known to colonize distilled liquors and alcohol-soaked swabs in numbers sufficient to cause infection.

2. Materials and Methods

Study Area

Uli town is located between latitudes 5.7833°N and 6.86071 in the South eastern part of Nigeria. Uli is predominantly a low-lying region on the Western plain of the Manu River with all parts at 333 meters above sea level. Uli has rainforest vegetation with two seasonal climatic conditions. They are the rainy and the dry season which is characterized by the harmattan between December and February, Uli is characterized by the annual double maxima of rainfall with a slight drop in either July or August known as dry spell or August break. The annual total rainfall is about 1600mm with a relative humidity of 80% at dawn. U has mean daily temperature of 18℃, annual minimum and maximum temperature ranges are about 22℃, and 34℃ respectively.

Sample Collection and Handling

A total of 100 samples of powdered soybean was collected from different shops and open markets; Amanputu market, Nkwoegbu, Aforegbu, and school market all within Uli community, using sterile polyethene bags and kept in disinfected cooler. A representative sample of commercially available powdered soybeans products was selected to ensure the study's findings are applicable to the broader population. The sampling process will involve random or stratified sampling, taking into account different brands, packaging types, production batches, and regions of distribution to capture the variability in fungal contamination levels (Samson, 2010). Proper aseptic techniques will be used during sample collection to prevent cross-contamination. The collected samples were processed within 2hours of its collection the samples were collected randomly. Laboratory analysis was performed using standard microbiological techniques and molecular methods for the detection and quantification of Bacillus cereus.

Isolation of organisms:

One gram of the sample was aseptically transferred into a sterile test tube (Pyrex) containing the diluent (sterile normal saline), this was thoroughly shake and made it up to 10 mL using the normal saline. One milliliter of the prepared sample was plated on Petri dishes (60 mm OD × 55 mm ID × 13 mm high) containing Nutrient agar medium (NA/Biotech) using pour plate method. All the plates in triplicates were incubated inverted at 37±2˚C for 24-48 h.

Characterization and identification of the isolates:

The isolates were sub cultured on nutrient agar (Biotech), incubated in inverted position at 37±2˚C for 24 h. The isolates were characterized and identified using their colonial and morphological descriptions as described in the study published by Iheukwumere *et al*. (2018), Ekesiobi *et al*. (2025a) and Ekesiobi *et al*. (2025b) biochemical reactions as described in the study published by Iheukwumere *et al*. (2021), Ekesiobi *et al*. (2025c) and Ekesiobi *et al*. (2025d) and molecular characterization as described in the study published by Gabriela *et al*. (2014), Ekesiobi *et al*. (2025e) and Ekesiobi *et al*. (2025f). The colonial description was carried out to determine the colours of the isolates on agar media plates, their sizes, edges, consistencies and optical properties of the isolates.

Prevalence and Distribution of the Isolates in the Samples

The number of each bacterial isolate in each sampling area was enumerated, and these were calculated as a percentage of the occurrences. The bacteria that appeared in each sample location were detected and recorded as described in the study published by Iheukwumere *et al*. (2021), Ekesiobi *et al*. (2025g) and Iheukwumere *et al*. (2025a).

Statistical Analysis

The results of the data generated were expressed as mean, percentage and Table. Data were analyzed by one-way Analysis of Variance (ANOVA) to determine the significance of the study at a 95 % confidence level. Pairwise comparison of means was done by Student “t” test as described in the study published by Iheukwumere *et al*. (2018), Iheukwumere *et al*. (2025b), Iheukwumere *et al*. (2025c), Iheukwumere *et al*. (2025d) and Iheukwumere *et al*. (2025e).

3. Results

The study revealed that 33% of the samples tested positive for *Bacillu*s species, with significant variation in occurrence across different locations ($p < 0.05$). Samples from Location

E had the highest occurrence of Bacillus species, while those from Location D had the lowest.

The isolates exhibited distinct appearances on Nutrient agar, with similar edge and surface characteristics. Gram reaction, cell morphology, endospore, and motility tests revealed consistent patterns among the isolates (Table 2). The isolates were characterized as methyl red, indole, urease, and D-mannitol negative. However, they were catalase, citrate, gelatin, Voges-Proskauer, oxidase, and hydrogen sulphate production positive. Variations were observed in sugar utilization patterns, particularly with lactose, sorbitol, and inositol (Table 3).

The nucleic acid extracts had an absorbance ratio of 1.82-1.83 at 260/280 nm, confirming the presence of DNA (Table 4). Molecular analysis identified two *Bacillus cereus* strains: FORC60 (BCFOR) and DQ01 (BCDQO) (Table 5). Notably, BCFOR occurred more frequently ($p < 0.05$) than BCDQO in the studied samples (Table 6).

These findings highlight the presence of *Bacillus cereus* in the samples, with BCFOR being the predominant strain. The study's results have implications for understanding the distribution and characteristics of Bacillus species in the studied samples.

Table 1: Occurrences of the isolates in the samples

Sample Location	**Number**	**P (%)**	**N (%)**
A	20	6(30.00)	14(70.00)
B	20	4(20.00)	16(80.00)
C	20	9(45.00)	11(55.00)
D	20	3(15.00)	17(85.00)
E	20	11(55.00)	9(45.00)
Total	100	33(23.00)	67(67.00)

Table 2: Cultural and morphological characteristics of the isolates

Parameter	Isolate M	Isolate N
Appearance on Nutrient Agar	cream white	white
Elevation	Flat	Flat
Edge	Irregular	Irregular
Surface	Rough	Rough
Gram Reaction	+	+
Cell morphology	Rods	Rods
Endospore	+	+
Position of spore	central	Central
Bulging	—	—
Motility	+	+

Table 3: Biochemical characteristics of the isolates

Parameter	Isolates M	Isolates N
Catalase	+	+
Citrate	+	+
Gelatin	+	+
Methyl red	–	–
Vogesprokauer	–	–
Indole	–	–
Oxidase	+	+
Urease	–	–
H_2S	+	+
Glucose	+	+
D-mannitol	–	–
Lactose	+/-	–
Sucrose	+	+
Maltose	+/-	+
Sorbitol	+/-	+
Inositol	+/-	+

Table 4: DNA quantifications of the nucleic acids from the isolates

Sample ID	Conc (mg/mL)	2600nm	280nm	260/280
M	166.40	3.5146	1.9312	1.82
N	172.20	3.6142	1.9750	1.83

Table 5: Molecular identities of the isolates

Parameter	M	N
Max Slave	7498	11501
Total Score	7498	11501
Query Cover (1%)	100	100
E-valve	0.0	0.0
Identity (900)	100	100
Accession Length	5361178	5322598
Accession Number	CP020 383	CP097351
Description	*Bacilluscereus* Strain FORC60 (BC FOK)	*Bacilluscereus* Strain DQ01 (BCDQO)

Table 6: Occurrences of the Isolate in the Sample

Isolates	Number	Percentage (10)
BCFOR	23	69.70
BCDQO	10	30.30
Total	33	100.00

4. Discussion

Bacillus cereus is a spore-forming bacterium commonly found in soil and various food products, including powdered soybean. The potential for *Bacillus cereus* to produce heat-stable toxins poses a significant risk of foodborne illnesses if contaminated products are consumed (Sonu *et al*., 2014). The present study focuses on the sectional Cross sectional studies on *Bacillus cereus* among powered soy beans sold at Uli Community, Ihiala L.G.A., Anambra State, Nigeria. The high occurrences of *Bacillus* species in the powdered soy bean samples revealed heavy contamination. This observation corroborates to the findings of several researchers (Ugwu *et al*., 2009; Odeyele *et al*., 20214; Ocholi, 2018) who evaluated microbial contamination in foods. However, the highest occurrence (33%) observed in this study was slightly lower than the highest occurrence (34%) reported by Ocholi (2018). This variation could be attributed to sources of contamination and storage conditions.

The cultural, morphological, and biochemical characteristics of *Bacillus cereus* in this study conform to the features reported by several researchers (Ugwu *et al*., 2009; Odeyele *et al*., 20214; Ocholi, 2018) who isolated and characterized *Bacillus* species in grains and powdered flours. The ability of the bacterial isolates to utilize common sugars such as glucose, sucrose, lactose, and maltose, and some sugar alcohols such as sorbitol and inositol, indicates their high enzyme-producing potentials. This ability had been described by other researchers (Ugwu *et al*., 2009; Odeyele *et al*., 20214; Ocholi, 2018). Molecular characterization of the bacterial isolates revealed the presence of two strains of *Bacillus cereus*, namely, *Bacillus cereus* strain FORC60 (BCFOR) and *Bacillus cereus* strain DQ01 (BCDQO). The occurrence of *Bacillus cereus* in strains had been reported by Chaves *et al*. (2011), Sampundo *et al*. (2011) and Sanchez-chica *et al*. (2020), who stated that molecular techniques provide a more sensitive and rapid technique for detecting bacterial pathogens.

5. Conclusion

This study highlights the significant presence of *Bacillus cereus* strains FORC60 and DQ01 in powdered soybean products, posing a potential risk to public health. Targeted preventive measures, consumer awareness, and adherence to safety practices are essential to minimize foodborne illnesses. Regulatory authorities should enforce safety standards to safeguard public health.

Acknowledgment

We are grateful to all our study participants who join the study voluntarily. We are grateful to ZAHARM Analytical and Research Laboratory, Amawbia, Awka Anambra State, Nigeria for providing enabling environment, resources and techniques for this study. We really salute their wonderful efforts.

Conflict of interests: The authors declare that they have no conflict of interests.

Funding: This research did not receive specific grant from any funding agencies.

Ethical approval: Not applicable

Authors Contributions: All contributed towards the study design, experiment execution, data analysis, and manuscript drafting.

Availability of Data and Materials: All datasets analyzed and described during the present study are available from the corresponding author upon reasonable request.

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