Original Research Article

ISOLATION AND IDENTIFICATION OF *BACILLUS CEREUS* AND *ESCHERICHIA COLI* FROM FOOD SAMPLE SOLD WITHING, KADUNA TOWN

Abstract
This study was carried out to isolate and identify *Bacillus cereus* and *Escherichia coli*. Isolation and identification of *B. cereus* and *E. coli* isolated from food samples sold from local food vendors within Kaduna town, Kaduna State. This was done using conventional standard method. The molecular characteristics were confirmed by the Polymerase Chain Reaction (PCR) technique. The result of the cultural and morphological characteristics of the isolates reveal that *B. cereus* is a Gram positive, rod shaped bacterium with dried pink background surrounded by egg yolk precipitate on MYP medium while *E. coli* is a Gram negative rod shaped bacterium with Flat colonies, green metallic sheen and mucoid colonies on Eosin Methylene Blue (
EMB) agar. The biochemical identification shows that *B. cereus* is positive to haemolysis, catalase, citrate, Vouges Proskauer, motility and spore test. While *E. coli* is positive to catalase, indole, methyl red and motility test and shows a green sheen characteristics on Eosin Methylene blue agar. The PCR shows band at 204bp amplicons of 16S rDNA primer targeting bacterial DNA templates V3 hyper-variable region for *B. cereus* and *E. coli* respectively. The amplified DNA was sequenced and BLAST and accession number of KY962911.1 and KY009556.1 were obtained for *B. cereus* and *E. coli* respectively
Key word: *Bacillus cereus, Escherichia coli* Isolation and Identification.

INTRODUCTION

Bacillus cereus has been implicated in food intoxication; it is an opportunistic human pathogen that has been reported to cause local and systematic infection. Food poisoning caused by *Bacillus cereus* occurs all year round without any ecological distribution (Rasko *et al.,* 2005). Literatures have associate *B. cereus* with diarrhea, emetic syndrome and fatal meningitis in humans. *Bacillus cereus* is a Gram positive, facultative anaerobic, rod shaped, motile, spore formers, catalase positive, beta-hemolytic and does not ferment mannitol (Rosenquist *et al.,* 2005). The Spore of Bacillus cereus are widely disseminated and can survive extreme environmental conditions for a long time, it has been recovered from samples of soils, dust, cereal, crops, dirt, and water (Rahimi *et al.,* 2013). *Bacillus cereus* has a growth temperature range from 10^{o}C – 48^{o}C, with

optimal growth between 28^{o}C and 35^{o}C, a PH values of 4.9 to 9.3 and water activities of 0.92 - 1.0 (Sibanda *et al.,* 2010)

Escherichia coli is a Gram-negative, facultative anaerobic, rod-shaped, coliform bacterium of the genus *Escherichia*, it is commonly found as a commensal organism in the lower intestine of warm-blooded animals including humans (Tenaillon *et al., 2010).* Although most strains of *E. coli* have not been associated with disease condition, some serotypes have been implicated in serious food poisoning, following ingestion of contaminated food substances. The harmless strains are part of the normal flora of the gut, and can benefit their hosts by producing vitamin K_2, and preventing colonization of the intestine by pathogenic bacteria (Yu *et al.*, 2014). *E. coli* is expelled into the environment with fecal matter and grows massively in the fecal matter under aerobic conditions for 3 days, but its numbers decline slowly afterwards. *E. coli* and other facultative anaerobes constitute about 0.1% of gut flora, and fecal–oral transmission is the major route through which pathogenic strains of the bacterium cause disease. This makes them potential indicator organisms to test environmental samples for fecal contamination (Tenaillon *et al.*, 2010*).*

The rate of food born diseases has post a major challenge in the public health sector therefore, this study was carried out to isolate and identify the major bacteria associated with food born diseases.

MATERIALS AND METHOD

Samples Collection

Food samples were randomly purchased from local food vendors within Kaduna town; samples bought were collected into sterile wide mouth universal bottles with tight-fittiing caps. The samples were transported in ice pack thermo-flask to the Medical Laboratory of the Department of Microbiology, Faculty of Science, Kaduna State University for isolation. Food samples purchased were raw meat, roasted meat (Suya), smoked fish, Jellof rice, Wara (Soya Cheese).

Isolation of *Bacillus cereus* and *E. coli*

Ten (10) grams of the different food samples were homogenized respectively with 90ml of 0.1% peptone water in a screw capped flasks by means of horizontal and vertical agitation for few minutes and incubated for 24 hours at 37^{oc}. After 24 hours clear supernatant of homogenized samples were each subcultured on freshly prepared plates of MacConkey, and Mannitol Egg Yolk Polymyxin B (MYP) agar plates (Servin 2014). All cultures were incubated for 24 hours at 37^{oc} (Rasko *et al.,* 2005)). The plates was observed and each positive plate, one to three discrete colonies of presumptive *Bacillus cereus* and *E. coli* were subcultured on nutrient agar and kept in the refrigerator for further confirmation of the identity of the organism (Cheesbrough, 2009).

Biochemical Tests for Identification of *Bacillus cereus* and *Escherichia coli*

Biochemical tests used to identify the isolates include catalase, haemolysis, indole production, methyl red, Vouges-proskauer test, citrate utilization, oxidase test, spore test and motility test (Holt *et al.*, 1993).

Molecular Identification

Molecular identification by the 16s RNA polymerase chain reaction

Molecular confirmation of isolates was determined according to the 16S rRNA gene region. The PCR amplifiability was checked using 16S ribosomal RNA primers V3F-CCAGACTCCTACGGGAGGCAG and backward V3R-CGTATTACCGCGGCTGTCTGG. Extraction was carried using Extraction buffer (Liferiver Biotech), using manufacturer's protocol. 50µl of the buffer was added to 50µl of isolate solution in molecular biology grade water, and incubated at 100^{o}C for 10minutes using a thermocycler (ABI, Proflex, Life Technologies) and centrifuge at 1300rmp. 5µl of the supernatant was used as template for Polymerase Chain Reaction **(**PCR) Five (5µl) bacterial Deoxyribo Nucleic Acid (DNA) extract and controls were amplified with 0.5mM primers using 5X Firepol Mastermix ready to load PCR kit (Solis Biodyne). Amplification conditions for PCR was as follows: 5minute at 94^{o}C to denature the DNA, followed by 30 cycles of denaturation at 94^{o}C for 30seconds, primer annealing at 55^{o}C for 40seconds and extension at 72^{o}C for 5minutes on a kyratech Supercycler Trinity thermal cycler. Polymerase Chain Reaction (PCR) fragments were separated on a 1% agarose gel and DNA bands were visualized with ethidium bromide product were quantified by comparing the intensity of the band to bands of known intensity in a 100Bp DNA Ladder (Promega). The gel was viewed under UV light after fluorescent dye staining. The ladder on the agarose gel indicated the base pair fragment of the DNA of the bacterial. Amplified Polymerase Chain Reaction (PCR) fragments were purified and sequenced with universal primers using Dye Terminator Cycle sequencing kit. Gene sequence chromatograms of the isolates were observed. . The similarity search was conducted in-silico using the Nucleotide Basic Local Alignment Search

Tool at the National Centre for Biotechnology Institute (NCBI) server. The phylogenetic and molecular evolutionary analyses were conducted using MEGA version 7 (Kumar, Stecher, and Tamura 2016) using the neighbor-joining method.

Statistical Analysis

All data obtained for this work were subjected to two-way analysis of variance (ANOVA), between data obtained using two way classification, least significant difference (LSD) test was

carried out at ($P<0.05$) to determine significant difference between the means as described by Mukhtar(2013)

Result and Discussion

Bacillus cereus is a common soil saprophyte and is easily spread to many type of foods such as dairy products, rice, cereals and cereals derivatives, dried foods, spices, eggs, vegetables and meats (Granum, 2005), it is also the causative agents of two forms of food poisoning; the diarrhoel form and an emetic form (Mahler *et al*., 1997), the wide spread of the organism and the ability of its spores to survive dried storage means that most raw and ready to eat foods may contain *B. cereus*.

Bacillus cereus was determined based on the colonial morphology, precipitation of hydrolyzed lecithin around colonies and its failure to utilize mannitol sugar as reported by Mosssel *et al*. (1967). Generally the colonies of *Bacillus cereus* on Mannitol Yolk Polymyxin B (MYP) appear rough and dry with a bright pink background surrounded by an egg yolk precipitate (Broth, 2014). *B. cereus* is a Gram positive (purple colored) on gram stain, rod shaped cells Abraha *et al*. (2017) reported similar findings of *B. cereus*. Biochemical characteristics of this organism were catalase positive, β-hemolysis on blood agar plate, does not ferment mannitol, motile, and spore-forming.

Escherichia coli are bacteria commonly found in the lower intestine of warm blooded organism. Most pathogenic *E.coli* is transmitted by fecal oral route from food materials, water, animals and environment. Food surfaces such as meat, eggs, or fish can be use to isolate *E.coli.* Phenotypic characterization revealed *Escherichia coli* ferment lactose on macConkey and produce pink, round medium sized colonies. *E. coli* appears green metallic sheen color colonies growth on Eosin Methylene Blue (EMB) agar plate. It

cellular morphology was revealed to be Gram negative short-rods due to the presence of thin layer of peptidoglycan that made it unable to retain the primary dye. The biochemical characteristic of *E. coli* was due to it cell wall component and enzyme activity. It was found to be methyl red, indole, citrate positive, and oxidase, and Voges-Proskauer (Whitman *et al.,* 2012). Holt *et al.,* (1993) have reported similar phenotypic characteristics. The bacterial species were identified as *Bacillus cereus* and *Escherichia coli* using both conventional and molecular methods. The amplified DNA was sequenced and BLAST and accession number of KY962911.1 and KY009556.1 were obtained for *E. coli* and *B. cereus* respectively.

The molecular characteristics were confirmed by Polymerase Chain Reaction (figure 1.**).** Bands shows 204bp amplicons of 16S rDNA primer targeting bacterial DNA templates V3 hyper-variable region. The bacterial species were identified as *Bacillus cereus* and *Escherichia coli* using both conventional and molecular methods as described in the methodology (Servin 2014). The amplified DNA was sequenced and BLAST and accession number of KY962911.1 and MG557810.1 were obtained for *E. coli* and *B. cereus* respectively

Table 1: Cultural, Cellular and Biochemical Characteristic of the Isolates

Cultural Morphology	**Cellular Morphology**	**Biochemical Characteristic**							**Most Probable Organism**
		He	**Ca**	**Ci**	**In**	**Mr**	**Vp**	**Ox**	
		Mt	**S**						
Rough, dried with pink background surrounded by egg yolk precipitate on MYP	Gram Positive rod	+	+	+	-	-	+	-	*Bacillus cereus*
		+	+						
Flat colonies with Green metallic sheen, mucoid Colonies	Gram negative rods	-	+	-	+	+	-	-	*Escherichia coli*
		+	-						

Key: **+**=Positive, **-** =Negative**, He**=Haemolysis, **Ca**=Catalase, **Ox**=Oxidase, **In**=Indole, **Mt**=Motility, **Mr**=Methyl Red, **Vp**=Vouges Proskauer, **S=**Spor

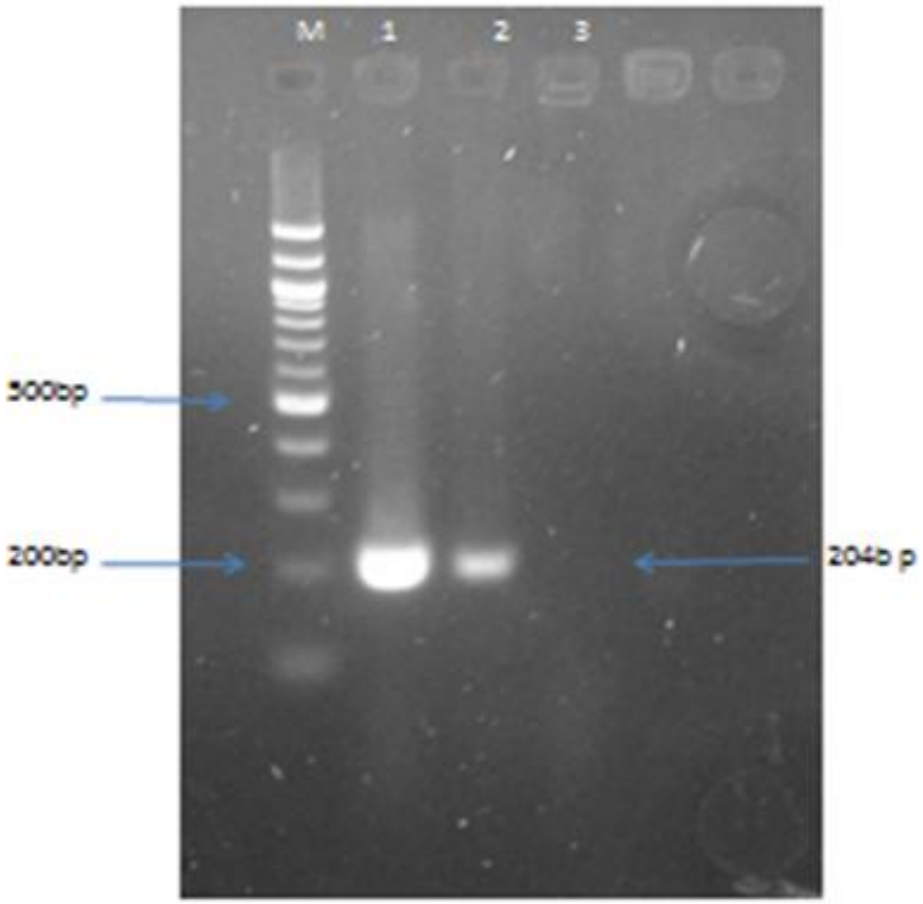

Figure 1: Agarose gel electrophoresis showing positive bands for *B. cereus* and *E. coli*

Table 2: Molecular Characterization of *Escherichia coli* and *Bacillus cereus*

Organism	Maximum Score	Total Score	Query Cover	E-value	Accession Number
Escherichia coli	307	307	99%	8e-80	KY962911.1
Bacillus cereus	1284	1284	100%	00-00	MG557810.1

Conclusion

Escherichia coli and *Bacillus cereus* were isolated from the food samples in a percentage frequency of 92% and 56% respectively

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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