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Full Length Research Paper

Toxin production in food as influenced by pH, thermal treatment and chemical preservatives

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Sixteen foods borne bacteria were isolated from raw food samples including okro, carrot, spinach, pepper, tomato, onion and cooked food samples (rice, yam, beans, meat and plantain). The isolates were characterized and identified as *Bacillus brevis*, *Bacillus congulans*, *Bacillus polymyxa*, *Bacillus lentus*, *Bacillus megaterium*, *Bacillus subtilis*, *Acinetobacter* spp., *Citrobacter freundii*, *Klebsiella aerogenes*, *Streptococcus agalactiae*, *Alcaligenes* spp., *Corynebacterium* spp., *Enterobacter aerogenes*, *Enterobacter* spp. and *Staphylococcus epidermidis*. These isolates were screened on egg yolk agar for toxigenic properties and thirteen of the sixteen were positive for toxin production while three were negative. Six out of the thirteen toxigenic bacterial were selected for further work. These were; *E. coli*, *K. aerogenes*, *C. freundii*, *B. polymyxa*, *S. epidermidis* and *E. aerogenes*. The effect of pH, thermal treatment and chemical preservatives on the growth rate and toxin elaboration of *E. coli*, *K. aerogenes*, *C. freundii*, *B. polymyxa*, *S. epidermidis* and *E. aerogenes* was studied. It was observed that *E. coli* had no viable growth until 48 h of incubation, while the other five isolates had visible growth right from the 24 h of incubation. Also *E. coli* did not produce toxin until the 96th hour of incubation; *K. aerogenes* and *E. aerogenes* were able to produce toxin at 24 h of incubation, while *C. freundii, B. polymyxa* and *S. epidermidis* produced toxin at 48 h of incubation. Also, 44°C was not suitable for toxin production. pH 3 and 5 were less favorable for toxin production despite the fact that isolates were able to grow at different temperature and pH ranges. The isolate were more sensitive to sodium metabisulfite than benzoic acid. Also, *E. coli* and *K. aerogenes* were able to elaborate toxin in their dormant state with 750 mg of sodium metabisulfite.

Key words: Toxin production, viable growth, raw food sample, cooked food sample, *E. coli, K. aerogenes*, *C. freundii*, *B. polymyxa*, *S. epidermidis* and *E. aerogenes*.

INTRODUCTION

Toxins are a variety substances that are toxic to eukaryotic cells (Orndoff, 1992), or a microbial substance that is able to induce host damage (Madigan et al., 1997), or any organic microbial product or substance that is harmful or lethal to cells, tissue cultures, or organisms (Atlas, 1995). It is important to note, however, that although many microorganisms secrete toxins that conform to these general criteria, only relatively few of them actually to influence the pathogenicity of the producing organisms (Williams and Clarke, 1998).

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It is striking that there is a close relationship between toxins produced not only by different groups of bacteria, but even by different microorganisms. The similarity between toxins produced by *Vibrio cholerae* and some strains of *Escherichia coli* has been known for some years. Related toxins may also be produced by *Salmonella* (Scotland, 1998). A cholera-like toxin is also produced by some strains of *Campylobacter jejuni* (Blazer et al., 1979) and, most remarkably, by the protozoan pathogen *Giardia lamblia* (Archer and Young, 1998).

The production of similar toxins by different microorganisms does not necessarily mean a similar role in pathogenicity. In most cases, toxin production is only a

part of the pathogenic process. However, with these food-borne pathogens, *Staphylococcus aureus*, *Bacillus cereus*, and under most circumstances, *Clostridium botulium*, toxins are preformed in the food and the presence of viable bacteria is not required in the disease condition (Varnam and Evans, 1991).

The toxins produced by microorganisms may be excreted into the surrounding medium (exotoxins) or retained within the cell (endotoxins) as part of the cell. Exotoxins are exracellular, that is, they diffuse freely into the medium in which the bacteria are growing and are easily separated from the cells by filtration. These toxins are active in small amounts. Examples of bacteria that produce exotoxins are *Corynebacterium diptheriae, Clostridium perfringes* and *Staphylococcus aureus* (Fruerst, 1983). The term enterotoxin, which is an exotoxin, implies that the poison acts on the intestinal mucosa in an abnormal way when the toxin is ingested or produced by an organism (Dupont, 1992)

Endotoxins are integral part of the cell and cannot be separated from the organisms by filtration. They are less active and usually do not stimulate antitoxin formation. Examples of bacteria that produce endotoxins are the typhoid *Bacillus* (*Salmonella typhii*), *Shigellia dysentariae*, and some a virulent strains of *Escherichia coli* (Fruerst, 1983).

In all, relatively few microbial toxins have been definitely established to be involved in disease and even fewer modes of action have been identified for these toxins. Exotoxin implies a protein that is found outside the bacteria cell, but while many exotoxins are indeed actively exported during growth, some such as cholera-like heat liable enterotoxin are only able to reach their site of action following bacterial death and lysis (Williams and Clarke, 1998).

In research laboratories, toxins have proved invaluable as tools to explore cellular physiology. Toxin which acts in the cytosol have been used as intracellular probes of metabolic activity such as protein synthesis and signalling pathways, while membrane acting toxins provide reagents for selectively alternating membrane composition and structure. A further practical outcome of such research may be toxin-based drug delivery systems for disease such as cancer where cell target specificity is essential. And finally, recent studies suggest that some toxin, such as those produced by *Bacillus thurigiensis* (Ellar, 1994) and species of *Cyanobacteria* (Sathiyamoorthy and Shanmugasundaram, 1996), may have potential value as biopesticides, providing safe, effective and cheaper alternatives to chemical pesticides in future (Williams and Clarke, 1998).

Over the years, raw and cooked food samples have been reported to be the vehicle for food-borne disease among in different countries as a result of contamination by pathogenic organisms (Boonestroo et al., 1993). The growth of these spoilage and toxigenic organism is therefore a potential hazard in foods that rely solely on mild heat treatment and refrigerated storage to maintain. Therefore, the objectives of this work are; Isolation and characterization of food-borne bacteria from raw and cooked food samples, screening for toxigenic strains amongst the isolates and determination of the effect of thermal treatment, pH and chemical preservatives on the growth response of the isolates as well as their toxin production in a selected food sample.

MATERIALS AND METHODS

Collection of food samples

Raw food samples which include okro, carrot spinach, pepper, tomato, onion were bought from Bodija Market, Ibadan, Nigeria, while cooked food samples including beans, yam, rice, plantain and meat were bought from medical student's food canteen, University of Ibadan, Nigeria. The food samples were aseptically transported to the laboratory.

Culture media

The culture media used in this study were Eosin methylene blue agar, MacConkey agar, Egg-yolk agar and nutrient agar. All the media were prepared according to manufacturers specification, and sterilization was done using the autoclave at 121°C (1.02 $kgcm^2$ pressure) for 15 min except where otherwise stated.

Isolation of microorganisms

10 g of each food samples were serially diluted and plated out in Eosin methylene blue agar and MacConkey agar. The plates were allowed to set and then incubated at 37°C for 24 – 48 h.

Isolation of pure culture

Different colonies obtained on the plates were transferred on to nutrient agar plates aseptically and incubated at 37°C for 24 h. Subsequent streaking was done, until pure cultures were obtained. The morphology of the colonies was observed using the microscope and the pure cultures were then inoculated on to agar slants and stored at 4°C.

Characterization of the Isolate

The routine laboratory methods of Cruickshank et al. (1975) and Sneath (1986) were used to characterize different isolates. The isolates were identified using their macroscopic, microscopic, physiology and biochemical characteristics.

Screening for toxigenic pathogens

This test was carried out to test the ability of the isolates to produce the enzyme lecithinase (it is also known as α-toxin) which reacts with lecithin in egg-yolk medium resulting in an opalescence which indicates lecithinase activity and iridescent layer which indicates lypolysis. The isolates were streaked on a single line unto the egg-yolk medium and incubated for 24 h at 37°C (Cruickshank et al., 1975).

Effect of incubation time on toxin production and growth response of the isolates

The food sample selected for monitoring toxin production was Ogi. 100 g of drained paste of Ogi was dissolved in 1 L of distilled water and 10 ml was dispensed in test tubes. The test tubes were sterilized, and then singly inoculated with isolates followed by incubation at 37°C for 5 days. Growth and toxin production was monitored at 24 h interval for 120 h. Growth was measured by viable count and toxin production was detected by lecithinase activity. This was done by centrifuging the already inoculated Ogi medium at 3,600 rpm for 15 min, and decanting supernatant. Holes were bored on egg yolk medium with 7 mm cork borer and a sterile Pasteur pipette was used to introduce few drops of the supernatant in the bored holes. It was then incubated for 24 h at 37°C (Rose et al., 1983 and Quinto and Cepeda, 1997).

Effect of temperature on toxin production

1 ml of inoculums of each isolate was introduced into test tubes, each containing 10 ml of Ogi broth. These were incubated at 20, 37 and 44°C for 14 days after which the tubes were centrifuged and the supernatant decanted and tested for toxin production. Viable count was determined on the fifth day of incubation to determine the growth response of the isolates (Kerstin et al., 1966; Carlin and peck, 1996).

Effect of PH on toxin production

Citrate phosphate buffer was prepared consisting of (a) 0.1 M citric acid in 1 L of distilled water and (b) 0.2 M Na_2HPO_4 in 1 L of distilled water. The two solutions were mixed in different proportions and added to the Ogi broth to obtain the desired pH values which were pH 2.0, 3.0, 5.0, 7.0, 8.0 and 9.0; 10 ml was dispensed in each test tube and sterilized. The tubes were then incubated with one millilitre of inoculum at 37°C for 14 days after which toxin production was tested by lecithinase activity. Growth was measured on the fifth day of incubation by viable count (Babara et al., 1990).

Effect of chemical preservatives on toxin production

The chemical preservatives used were benzoic acid and sodium metabisulfite. 250, 500 and 750 mg concentrate of the chemical preservatives per liter of Ogi broth was used. These were dispensed in test tubes in 10 ml per tube and were sterilized. 1 ml of the inoculums was inoculated into the tubes, and then incubated at 37°C for 14 days, after which toxin production was tested by lecithinase activity. Growth response of the isolates was determined on the fifth day of incubation (Babara et al., 1990).

RESULTS

The result of isolation obtained showed that none of the food samples used for this study was completely sterile; they were colonized by bacteria of different groups. From okro, four organisms were isolated, four from spinach, four from beans, two organisms from carrot, two from plantain, two from pepper, two from tomato, two from onion and two from meat, also one organism was isolated from rice and one from yam (Table 1). Morphological and biochemical characterization of the isolates showed that they consist of both Gram-positive and Gram-negative bacteria. The bacteria isolated include 6 species of *Bacillus*, 2 species of *Enterobacter*, *Acinetobacter*, *Citrobacter*, *Klebsiella*, *Streptococcus*, *Alcaligenes*, *Corynebacterium*, *Escherichia* and *Staphylococcus* (Table 1).

Furthermore, the isolates were screened on egg-yolk agar for toxin production. Only 3 out of 16 isolates were negative and these include *Corynebacterium* spp., *Enterobacter* spp. and *Bacillus megaterium*, while the remaining 13 isolates were positive for toxin production. There were also some strains of the 13 isolates that were negative e.g. out of 3 strains of *B. lentus* only one was positive while the remaining 2 were negative, one strain of *B. coagulans*, one of *B. subtilis,* one of *Citrobacter freundii*, one of *K. aerogenes* and one strain of *Alcaligenes* spp. Were negative for toxin production (Table 1). Out of the 13 toxigenic bacteria, 4 enteropathogens: *E. coli*, *Klebsiella aerogenes, Citrobacter freundii, Enterobacter aerogenes* and 2 opportunity pathogens: *Bacillus polymyxa* and *Staphylococcus epidermidis* were selected for further work.

When the effect of incubation time on growth response of isolate was monitored, it was found out that at 24 h, *E. coli* produced no visible growth, only to produce growth at 48 h, the growth decrease at 72 h to increase at 96 h and decrease at 120 h. At 24 h, the other 5 isolates produce visible growth except that the number of colonies had decrease compare to the initial size of inoculum. The effect of incubation time on toxin production showed that at 24 h, *K. aerogenes* and *E. aerogenes* were able to produce toxin. *E. coli* did not produce detectable toxin until 96 h while *C. freundii, B. polymyxa* and *S. epidermidis* produced toxin at 48 h (Tables 2 and 3).

Figure 1 shows the growth response of isolates to temperature and it was observed that all the isolates were able to grow and survive at 20, 37 and 44°C. Also, the results of the effect of temperature on toxin production were summarized in Table 4. It was found that at 20°C, *E. coli* and *B. polymyxa* failed to produce toxin while other isolates produced toxin. All the isolates produced toxin at 37°C while only *E. aerogenes* produced toxin at 44°C.

The results of the effect of pH on the growth of isolates are shown in Table 5. All the isolates were able to grow at the various pH values. It was noted that the viable count (log_{10} cfu/ml) of *E. coli* was reducing as the acidity of the medium reduced; *E. coli* had the high viable count 6.68 at pH 9. *K. aerogenes* viable count was observed to reduce as the acidity of the medium was reducing and as alkalinity was increasing. *B. polymyxa*, *S. epidermidis* and *E. aerogenes* had highest viable count (6.76, 6.71 and 6.69 respectively) at pH 3. While *C. freundii* was also observed to have viable count that reduced as acidity was also reducing. Table 6 shows that all the isolates were able to produce toxin at pH 2 and 7. At pH 3, only *C. freundii* and *E. aerogenes* were able to produce toxin.

Table 1. Bacteria isolated from food samples and screened for toxigenic property.

Isolates	Source	Lecithinase activity
Bacillus brevis	Okro	+
Acinetobacter spp.	Okro	+
Citrobacter freundii	Okro	+
Klebsiella aerogenes	Okro	+
Acinetobacter spp.	Carrot	+
Streptococcus agalactiae	Carrot	+
Acinetobacter spp.	Plantain	+
Alcaligenes spp.	plantain	+
Corynebacterium spp.	Spinach	-
Bacillus coagulans	Spinach	-
Eschrichia coli.	Spinach	+
Citrobacter freundii	Spinach	-
Enterobacter spp.	Beans	-
Bacillus magaterium	Beans	-
Bacillus subtilis	Beans	-
Klebsiella aerogenes	Beans	-
Enterobacter aerogenes	Pepper	-
Enterobacter aerogenes	Pepper	+
Bacillus coagulans	Pepper	+
Bacillus subtilis	Tomatoe	+
Bacillus lentus	Tomatoe	-
Bacillus lentus	Onion	+
Alcaligenes spp.	Onion	-
Staphylococcus epidermidis	Meat	+
Bacillus polumyxa	Meat	+
Alcaligenes spp.	Yam	+
Bacillus brevis	Rice	+

Table 2. Viable count (log_{10} cfu/ml) of test isolates obtained from food samples.

Isolate	Time (h)					
	0	24	48	72	96	120
E. coli	6.66	ND	6.15	6.34	6.66	6.30
K. aerogenes	6.48	6.34	6.51	6.36	6.69	6.69
C. freundii	6.62	6.48	6.31	6.60	6.59	5.81
B. polymyxa	6.63	6.45	6.61	6.68	6.63	6.62
S. epidermidis	6.57	6.43	6.66	6.58	6.49	6.49
E. aerogenes	6.61	6.27	6.27	5.79	6.31	6.02

ND = Growth not detected.

At pH 5, *B. polymyxa* and *S. epidermidis* were unable to produce toxin at pH 8 while it was only *E. coli* that was unable to produce toxin at pH 9 while the other isolates produced toxin.

Figure 2a shows the result of the effect of benzoic acid on the growth of the isolates. All the isolates were noted to grow in various concentrations of benzoic acid i. e. 250, 500 and 750 mg/L of ogi. Viable count for *E. coli* was found to decrease as the concentration of benzoic acid was increasing (from 6.75 to 6.44). Also, *K. aerogenes* viable count decrease from 6.61 to 5.73. The same trend was noted with all the isolates except for *C. freundii* and *E. aerogenes*. In 250 mg of benzoic acid per litre of ogi, all the isolates were able to produce toxin, in 500 mg of benzoic acid per litre of ogi only *K. aerogenes* and *C. freundii* were unable to produce toxin, and in 750

Table 3. Effect of incubation time on toxin production by bacteria isolated from food samples.

Isolate	Time (h)					
	0	24	48	72	96	120
E. coli	ND	ND	ND	ND	+++	+++
K. aerogenes	ND	+++	+++	+++	+++	+++
C. freundii	ND	ND	+++	+++	+++	+++
B. polymyxa	ND	ND	+++	+++	+++	+++
S. epidermidis	ND	ND	+++	+++	+++	+++
E. aerogenes	ND	+++	+++	+++	+++	+++

+++ = Toxin detected; ND = not detected.

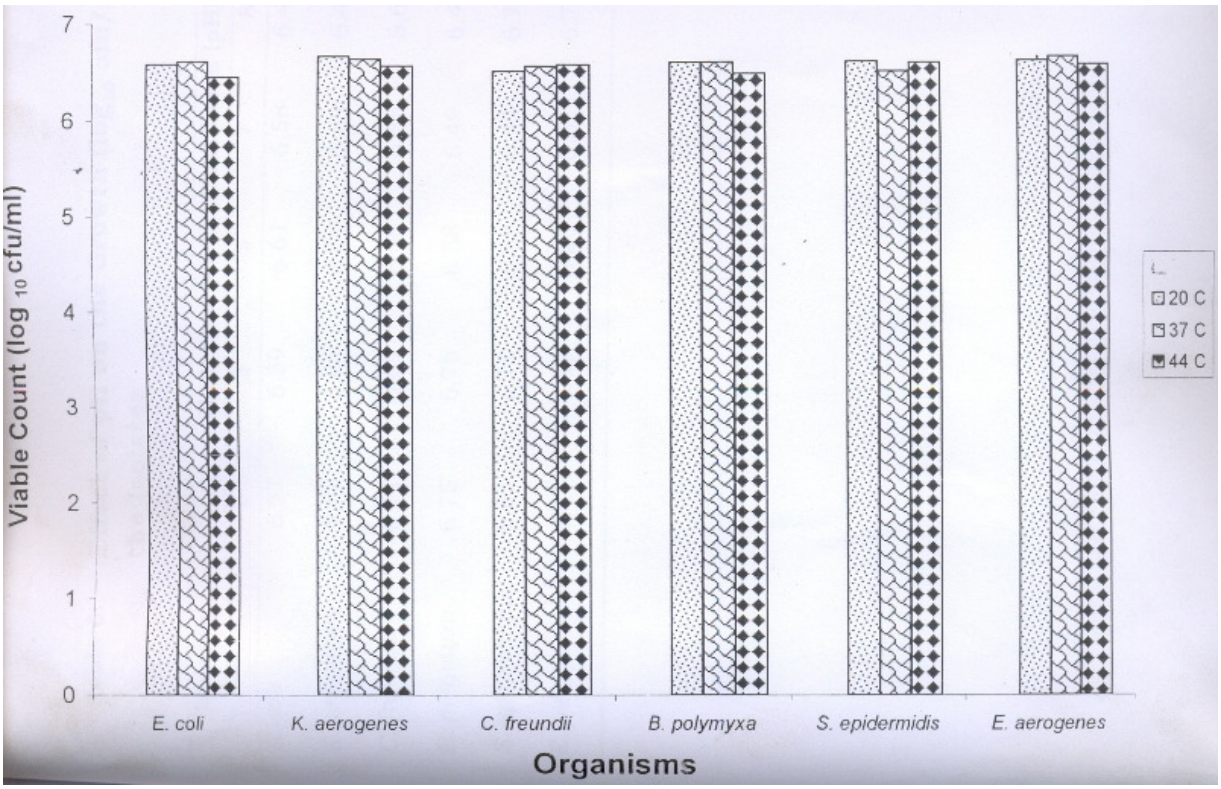

Figure 1. Effect of temperature on the growth (log_{10} cfu.ml) of the isolates.

Table 4. Effect of temperature on toxin production by bacteria isolated from food samples.

Isolate	Temperature (°C)		
	20	37	44
E. coli	ND	+++	ND
K. aerogenes	+++	+++	ND
C. freundii	+++	+++	ND
B. polymyxa	ND	+++	ND
S. epidermidis	+++	+++	ND
E. aerogenes	+++	+++	+++

+++ = Toxin detected; ND = not detected.

Table 5. Effect of pH on the growth (log_{10} cfu/ml) of the bacteria isolated from food samples.

Isolate	pH					
	2	3	5	7	8	9
E. coli	6.57	6.59	6.61	6.58	6.48	6.68
K. aerogenes	6.62	6.61	6.60	6.59	6.45	6.37
C. freundii	6.71	6.69	6.56	6.56	6.61	6.53
B. polymyxa	6.75	6.76	6.58	6.49	6.49	6.57
S. epidermidis	6.61	6.71	6.68	6.45	6.76	6.58
E. aerogenes	6.61	6.69	6.66	6.61	6.75	6.61

Table 6. Effect of pH on toxin production by bacteria isolated from food samples.

Isolate	pH					
	2	3	5	7	8	9
E. coli	+++	ND	ND	+++	+++	ND
K. aerogenes	+++	ND	ND	+++	+++	+++
C. freundii	+++	+++	ND	+++	ND	+++
B. polymyxa	+++	ND	+++	+++	ND	+++
S. epidermidis	+++	ND	+++	+++	+++	+++
E. aerogenes	+++	+++	ND	+++	+++	+++

+++ = Toxin detected; ND = not detected.

mg of benzoic acid per litre of ogi, also only *K. aerogenes* and *C. freundii* were unable to produce toxin (Table 7).

In 250 and 500 mg of sodium metabisulfite per litre of ogi all the isolates were able to grow but in 750 mg of sodium metabisulfite per litre of ogi, *E. coli, K. aerogenes* and *E. aerogenes* were unable to grow. It was observed that for most of the isolates as the concentration of sodium metabisulfite was increasing in the medium, viable count reduced (Figure 2b). The effect of sodium metabisulfite on toxin production was carried out (Table 8). It was observed that in 250 mg of sodium metabisulfite per litre of ogi, *C. freundii* and *E. aerogenes* were able to produce toxin, in 500 mg/L, *E. coli* and *K. aerogenes* produced toxin while in 750 mg of sodium metabisulfite per litre of ogi, *E. coli*, *K. aerogenes* and *C. freundii* produced detectable toxin.

DISCUSSION

The bacteria load of raw and cooked food samples examined showed that none of the food samples were sterile. The food samples were found to consist of different group of bacteria and this agreed with the work of Bonestroo et al. (1993) who reported that many spoilage and pathogenic bacteria were isolated from raw vegetable salads and ready-to-eat-food. It was also noted that contamination of the raw vegetables can occur in the field or during post harvest handling when contamination of cooked food might be due to inadequate cooking or improper storage of food as well as poor sanitary condition in food preparation.

It was observed from the result of screening on egg-yolk medium for toxigenic bacteria, that thirteen out of the sixteen isolates were positive for toxin production. This specific test has been used by many workers to test for toxigenic isolates (Cruickshank et al., 1975; Rose et al., 1983). Hence, the presence of those toxigenic organisms implies that there must have been toxin production in the food samples tested and had rendered them detrimental and hazardous to consumer's health.

The results of effect of incubation time on toxin production showed that *E. coli* was unable to produce toxin until the 96th hour, and this could be as a result of lack of proliferation before the 96th hour. Bonestroo et al. (1993) reported that some pathogen when inoculated can survive some time but cannot proliferate. However, Williams and Clarke (1998) observed that toxin can be released during proliferation. Thus, toxin production was observed at the 96th hour of incubation because at about the same time proliferation occurred. This explanation applies to *C. freundii*, *B. polymyxa* and *S. epidermidis*. *K. aerogenes* and *E. aerogenes* produced detectable toxin at 24 h of incubation.

The test carried out to determine the effect of temperature on toxin production showed that 44°C was not suitable for toxin production for nearly all the isolates, despite the fact that all the isolates were able to grow at

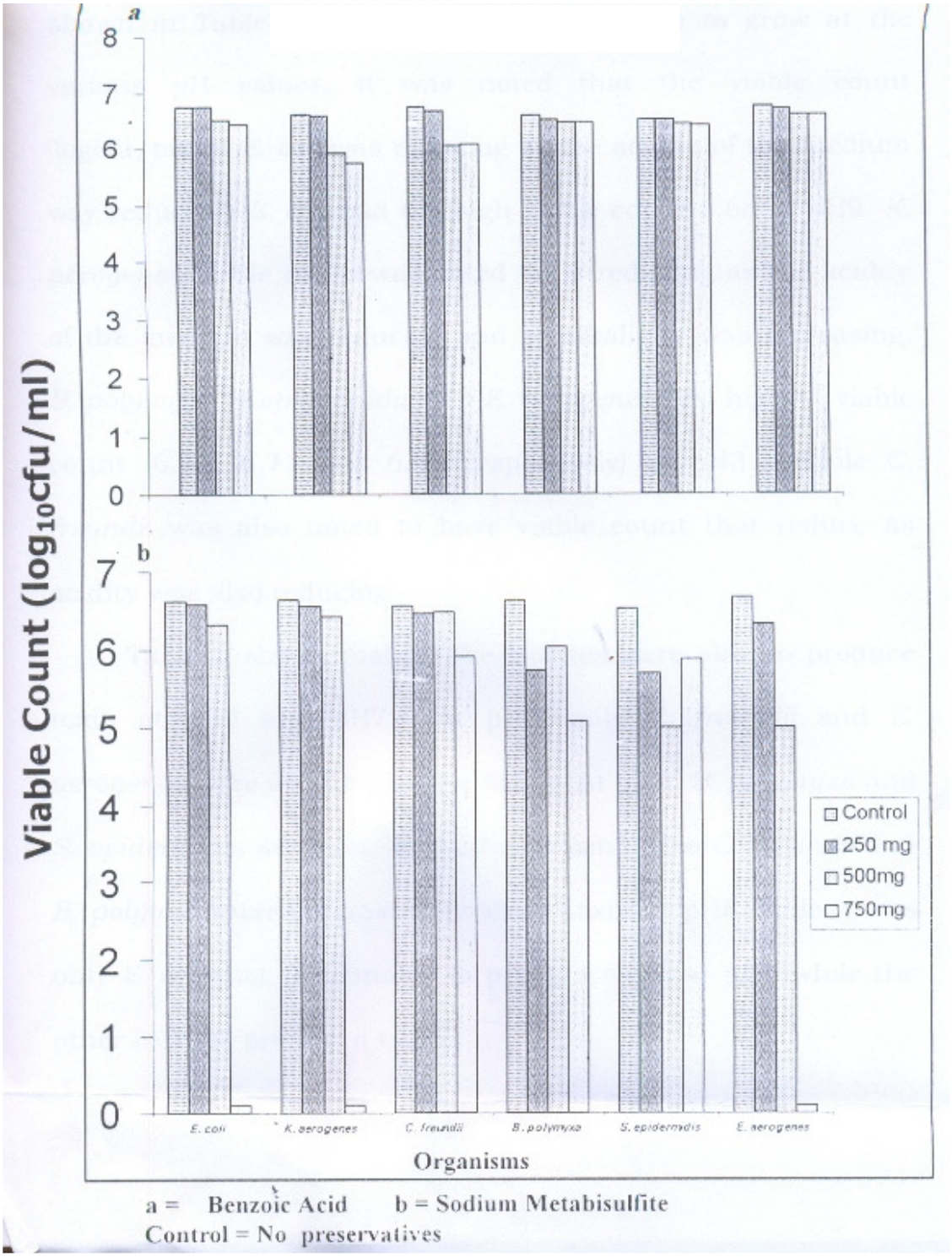

Figure 2. Effect of Chemical Preservatives on the Growth (log_{10} cfu/ml) of Isolates.

44°C. Also, *E. coli* and *B. polymyxa* did not produce toxin at 20°C while all the 6 isolates produced toxin at 37°C. This is in accordance with the studies of Perderson (1955) who reported formation of toxin at 37°C. It can also be explained further that 20 and 44°C were suitable for growth but not comfortable for elaboration of toxin for nearly all the isolates.

It was noted that at pH 2 and 7 all the isolates produced toxin, at pH 9 all except *E. coli* produced toxin. Despite this, all the isolates were able to grow at all the

Table 7. Effect of benzoic acid on toxin production by bacteria isolated from food samples.

Isolate	Concentration (mg/L of ogi)			
	0	250	500	750
E. coli	+++	+++	+++	+++
K. aerogenes	+++	+++	ND	ND
C. freundii	+++	+++	ND	ND
B. polymyxa	+++	+++	+++	+++
S. epidermidis	+++	+++	+++	+++
E. aerogenes	+++	+++	+++	+++

+++ = Toxin detected; ND = not detected.

Table 8. Effect of sodium metabisulfite on toxin production by bacteria isolated from food samples.

Isolates	Concentration (mg/L of ogi)			
	0	250	500	750
E. coli	+++	ND	+++	+++
K. aerogenes	+++	ND	+++	+++
C. freundii	+++	+++	ND	+++
B. polymyxa	+++	ND	ND	ND
S. epidermidis	+++	ND	ND	ND
E. aerogenes	+++	+++	ND	ND

+++ = Toxin detected; ND = not detected.

pH ranges.

The test of effect of chemical preservatives on toxin production shows that in 250 mg of benzoic acid per litre of ogi, all the isolates produced toxin but in 500 and 750 mg of benzoic acid per litre of ogi, *K. aerogenes* and *C. freundii* were unable to produce toxin while the other isolates produced toxin. This could be that the other isolates could tolerate high concentration of benzoic acid while *K. aerogenes* and *C. freundii* could not tolerate high concentration; this was also reflected in their growth, with *K. aerogenes* having the lowest count.

The isolates were more sensitive to sodium metabisulfite. In 250 and 500 mg of sodium metabisulfite per litre of ogi, 2 isolates were able to produce toxin while in 750 mg of sodium metabisulfite per litre of ogi, 3 isolates produced toxin. This was also reflected in their growth; in 250 and 500 mg of sodium metabisulfite per litre of ogi, all the isolates were able to grow while in 750 mg of sodium metabisulfite per litre of ogi, viable count for *E. coli*, *K. aerogenes* and *E. aerogenes* was not detectable or they had zero viable count. It can be found that *E. coli* and *K. aerogenes* did not produce viable growth but were able to produce toxin. It could be that the two isolates were dormant in the culture medium, but in their dormant state were able to elaborate or elicit their toxin.

Because of public health concern over possible health hazard posed by food additives and suspicion that poor hygiene and sloppy manufacturing practices are masked by the addition of preservatives to food products, most regulatory authorities oppose extension of the use of food preservatives. The preservatives used in this study, benzoic acid and sodium metabisulfite, were used at a concentration that is Generally Regarded As Safe (GRAS); 0.05 - 0.1%.

From the results obtained in this study and based on other observations, the isolation of these toxigenic bacteria calls for public health concern. If a toxigenic pathogen is present and its growth in the contaminated food is left unchecked, this contaminated specimen could very well become a vehicle involved in a food poisoning out-break. Therefore, the importance of proper handling and storage of food as well as the need to control growth of toxigenic pathogen needs to be emphasized.

REFERENCES

Archer DL, Young FE (1980). Contemporary issues; Diseases with a food vector. Clin. Microbiol. Rev., 1: 377-398.

Atlas RM (1995). Principles of microbiology. St. Lour. Mosby-year Book Inc.

Babara ML, Ann FG, Susan MG, Brown D (1990). The combined effect of incubation temperature pH and sorbic acid on the probability of growth of non-protelytic, type B clostridium botulinum J. Appl. Bacteriol. 69: 481-492.

Blazer, M.J., Berkowitz, I.D., Laforce, F. (1979). Campylobacter enteritis. Clinical and epidemiologic features. Ann. Int. Med. 91: 179-185.

Bonestroo MH, kirsters BJM, Dewir JC, Rombouts FM (1993). The fate of spoilage and pathogenic Bacteria in fermented sauce-based salads. J. Food Microbiol. 10: 101-111.

Carlin F, Peck MW (1996). Growth of toxin production by Non protolytic Clostridium botulinum in cooked pureed vegetables at Refrigerated Temperatures. J. Appl. Environ. Microbiol. pp. 3069-3079.
Cruickshank RC, Duguid BP, Marion BP, Swain RHA (1975). Medical microbiology 12th Ed. Vol. 11 Churchill living stone publishers Edinburgh London and New York, p. 186.
Dupont HL (1992). Enteropathogens. In Encydopedia of microbiology. Academic press Inc. 2.63-68.
Ellar DJ (1994). Structure and mechanism of action of bacillus thuringiensis endotoxins and their receptors. Bi Contr. Sci. Technol. 4: 445-447.
Fruerst R (1983). Microbiology in health and disease 5th ed. p. 282-283.
Kerstin A, Gullmar B, Molin N (1966). The effect of temperature on toxin formation and toxin stability of clostridium type E. in different environments. Can J. Microbiol. 12: 385-395.
Madigan MT, Martinko JM, Parker J (1997). Microbiology of microorganisms, 8th ed. London prentice – Hall international (UK) Ltd.
Orndoff PE (1992). Bacterial virulence: In the prokaryotes 2nd edn. Ed. Balows A, Truper HG, Dworkin M, Scliefer KH New York: Springer-Verlag. pp. 640-658.
Perdersen HO (1955). On type E. botulism. J. Appl. Bacteriol. 18: 619-629.
Quinto EJ, Cepeda A (1997). Incidence of toxigenic Escherichia coli in soft cheese made with raw or pasteurized milk. Lett. Appl. Microbiol 24: 291-295.
Rose NR, Barron AL, Crane LR, Menna JH (1983). Microbiology: Basic principles and Clinical Application P. 121-122. Macmillan pub.Com. New York.
Sathiyamoorthy P, Shanmugasundaram S (1996). Preparation of cyanobacterial peptide toxin as a biopesticide. J. Appl. Microbiol. Biotechnol. 46: 511-513.
Scotland SM (1988). Toxins. J. Appl. Bacteriol. 5: 1058-1295.
Sneath RHA (1986). Classification of microorganisms. In: Essays in microbiology. Norris JR, Richman MH (eds.) Wiley London.
Varnam AH, Evans MG (1991). Food poisoning, medical and microbiological overview. Food Borne Pathogens, pp. 9-140.
Williams PH, Clarke SC (1998). Why do microbes have toxins? J. Appl. Microbiol. Symp. Suppl. 84: l 8-6s.