



Growth and Survival of Enterotoxigenic *Escherichia coli* (ETEC) Isolated from Tuna Loins Produced in Côte D'ivoire

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Authors' contributions

His work was carried out in collaboration among all authors. Author AES designed the work, wrote protocol, carried out the experiments and wrote the first draft of manuscript. Authors LO and DTY analyzed data obtained and carried out literature searches. Authors RKN and KMD read and approved the final manuscript. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJPR/2020/v4i430117

Editor(s):

(1) Dr. Khadiga Ahmed Ismail Eltris, Ain Shams University, Egypt.

Reviewers:

(1) Ami Patel, Kamdhenu University, India.

(2) Mahmud Yerima Iliyasu, Abubakar Tafawa Balewa University, Nigeria.

Complete Peer review History: <http://www.sdiarticle4.com/review-history/58250>

Original Research Article

Received 17 April 2020

Accepted 23 June 2020

Published 04 July 2020

ABSTRACT

Aims: The aim of this study was to study the growth of pathogenic strains of *Escherichia coli* (ETEC) in Tuna loins.

Study Design: Bacteriological study.

Place and Duration of Study: Laboratory of Microbiology of the Central Laboratory of Food hygiene and Agro-business (LCHAI), Abidjan, Côte d'Ivoire between September 2014 and December 2014.

Methodology: Three strains of *E. coli* (enterotoxigenic strain of *E. coli* (ETEC), possessing both "elt" and "est" virulence genes resistant to amoxicillin from Tuna loins; *E. coli* reference strain (ATCC 25992); strain of *E. coli* (KO 13) from water with the virulence gene "elt") were inoculated in brain heart infusion broth (BHI) and in tuna loins for 120 hours. pH and bacterial loads of *E. coli* were measured to 0; 3; 6; 12; 24; 48; 72; 96 and 120 hours respectively.

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Results: The results showed that the three strains of *E. coli* used in this work survived in liquid medium (BHI) and in Tuna loins even after 5 days (120 hours). The growth curves of these three strains evolved in the same way in liquid medium and in Tuna loins. However, the growth rate of strains of *E. coli* inoculated in liquid media (BHI) was higher than that of strains inoculated in Tuna loins.

Conclusion: Pathogenic strains of *E. coli* isolated from Tuna loins are a hazard to be considered in the microbiological risk assessment of the consumption of these Tuna products.

Keywords: *Enterotoxigenic Escherichia coli (ETEC); tuna loins; Brain Heart Infusion broth (BHI); growth.*

1. INTRODUCTION

Escherichia coli are known a facultative anaerobic bacterium found in the normal flora of the intestinal tract of humans and most homeothermic (warm-blooded) animals [1]. Although most *E. coli* strains are commensal, some of them are associated with very diverse intestinal or extra-intestinal pathologies in humans [2]. These strains responsible for diarrhoea include several pathogens emerging around the world. They are important in public health because they have been associated with travelers' diarrhoea around the world in recent years [3;4]. In human medicine, based on these host-bacteria interaction modes and clinical signs of infection, the pathogenic *E. coli* strains are classified into "pathovars" or "pathotypes" which group together strains of specific serotypes [5].

The Enterotoxigenic *Escherichia coli* (ETEC) strains are mainly associated with two important clinical syndromes, choleraform watery diarrhoea in children called infant diarrhoea and traveler's diarrhoea (or "turista") in developing countries [6]. In these countries, in fact, almost 700,000 child deaths are linked to ETEC. Humans are reservoirs for strains of ETEC that can be transmitted to other humans through water or food contaminated with faeces [7]. The pathogenic power of ETEC is mainly explained by the secretion of thermostable (ST) and / or thermolabile (LT) toxins [8]. ETEC causes watery diarrhoea, generally feverless, non-bloody, mucous-free, with nausea, abdominal cramps [9]. Côte d'Ivoire through the processors and exporters of fish products, has become one of the largest exporters of tuna products to the global level [10]. There are 2 types of Tuna products exported: Tuna finished products (canned) and Tuna semi-finished products (tuna loins, tuna flakes, tuna skin and tuna pulp). The Tuna loins are portions of the tuna flesh usually skinless and boneless and ready to use. However, industries have difficulties to export

Tuna loins because they don't satisfy the criteria for hygienic quality and existing standards always. ETEC has been found in these products [11], which poses a major public health problem and causes economic losses for companies producing Tuna products. The study is aimed at monitoring the growth of pathogenic strains of *Escherichia coli* (ETEC) in Tuna loins.

2. MATERIALS AND METHODS

2.1 Sample Preparation

Each sample of Tuna loins was crushed and aseptically distributed in Pyrex bottles then sterilized at 121°C for 15 min. Each sample was approximately 100 g in each bottle.

Brain heart infusion broth (BHI) (Biorad, France) was prepared in accordance with the manufacturer's instructions and distributed in Pyrex bottles then sterilized at 121°C for 15 min. The volume of each broth was also 100 mL in each bottle.

2.2 Inoculum Preparation

Three strains of *E. coli* were selected for the various analyzes:

- an enterotoxigenic strain of *E. coli* (ETEC), possessing both the "elt" and "est" genes resistant to amoxicillin, isolated from Tuna loins;
- an *E. coli* reference strain (ATCC 25992);
- a strain of *E. coli* (KO 13) from water with the virulence gene "elt".

A colony of each strain was inoculated into 10 mL of Tryptone Soya Broth (TSB) (Mast Diagnostic, France) broth and incubated at 37°C for 24 hours. The optical density of the inoculated broth was determined using a UV 2700

spectrophotometer (Schimadzu, Germany) at a wavelength of 600 nm. Knowing that the absorbance between 0.5 and 1 corresponds to approximately 10^8 CFU/ml, the different concentrations of *E. coli* have been determined and the cultures diluted to obtain a final concentration of 10^5 CFU/ml.

2.3 Growth of *Escherichia coli* Strains

The study of the growth of *Escherichia coli* strains isolated from Tuna loins was carried out according to the methods described by [12]. 1 ml of each bacterial culture with a concentration of 10^5 CFU / ml was inoculated into 100 g of Tuna loins and 100 ml of BHI. The inocula were incubated at 30°C for 120 hours. At each time interval, 1 mL of inocula was withdrawn to determine the pH using pH meter (MILWAUKEE, USA) and 1 ml of the stock solution and these successive decimal dilutions were used to determine *Escherichia coli* on Tryptone Soya Agar (TSA) (PLASMATEC, ENGLAND) at 37°C for 24 hours. The measurement interval was (hours): 0; 3; 6; 12; 24; 48; 72; 96; 120.

2.4 Expression of Results

Growth capacity, specific growth rate and generation time have been calculated using [13].

2.5 Study of Growth Capacities

Growth capacity (CC) is defined as the difference in *E. coli* population between the start and the end of the preservation process.

$$CC = \log (N_{tf}) - \log (N_{ti}) \quad (2.1)$$

N = concentration of *E. coli* (CFU / g or CFU/ mL)
tf = final time (hours)
ti = initial time (hours)

2.6 Evaluation of the Bacterial Population as a Function of Time

The exponential model was used to evaluate the bacterial population as a function of time according to the following formula:

$$N(t) = N_0 \mu t; \ln N(t) = \ln N_0 + \mu t \quad (2.2)$$

$$\mu = \frac{\ln(N_2) - \ln(N_1)}{t_2 - t_1} \quad (2.3)$$

$$G = \frac{\ln(2)}{\mu} \quad (2.4)$$

N = Concentration of *E. coli* (CFU / g or CFU/ mL)

N₀ = Initial concentration of *E. coli* (CFU / g or CFU/ mL)

μ = Specific growth rate (h⁻¹)

t = time (hours)

G = generation time (minutes) = doubling time of the bacterial population

2.7 Statistical Analysis

All values were expressed as the mean of three measurements in microbiological analysis. *E. coli* counts were log transformed (Log₁₀) and the data collected were subjected to one ways Analysis of variance (ANOVA) with the software Statistica 7.1. Duncan test was used to determine which means were significantly different from which others (α=0.05).

3. RESULTS

3.1 Monitoring the Growth in Non-renewed Liquid Medium of *Escherichia coli* Strains

Figs. 1a to 1c show the monitoring in BHI on growth of strains of *E. coli* ATCC 25922, *E. coli* KO 13 and pathogenic *E. coli* isolated from Tuna loins respectively. The pH decreased from 7.22 to 5.84; 7.22 to 5.80 and 7.24 to 5.82 in BHI inoculated with *E. coli* ATCC 25922, *E. coli* KO 13 and pathogenic *E. coli* strains isolated from Tuna loins respectively. The bacterial loads increased over time from 3.11 to 9.71 log CFU/mL (Fig. 1a); 3.38 to 9.62 log CFU/mL (Fig. 1b) and 3.30 to 9.41 log CFU/mL (Fig. 1c). The curves showing the evolution of bacteria are characterized by two phases: an exponential growth phase from 0 to 24 hours and a stationary phase from 24 hours.

Fig. 1d shows the growth of the three strains of *Escherichia coli* studied. The three curves have the same appearance with values close to each other. They are characterized by two phases which present the physiological characteristics of the strains studied: an exponential phase translating a rapid growth from 0 to 24 h and a stationary phase from 24 h.

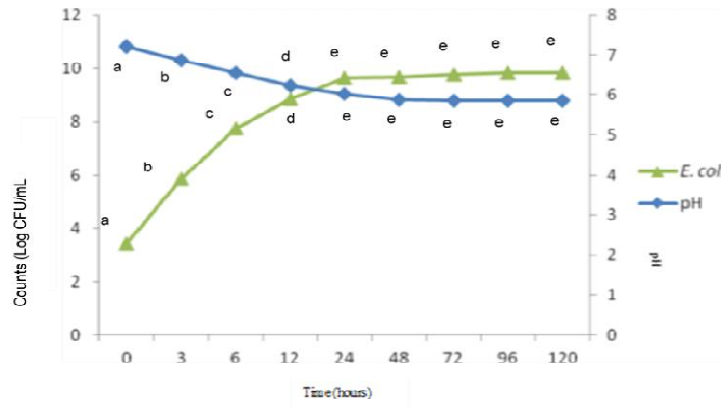


Fig. 1a. Evolution in BHI of pH and *Escherichia coli* ATCC 25922
 Values with the same letter on a curve are not significantly different for $p>0.05$

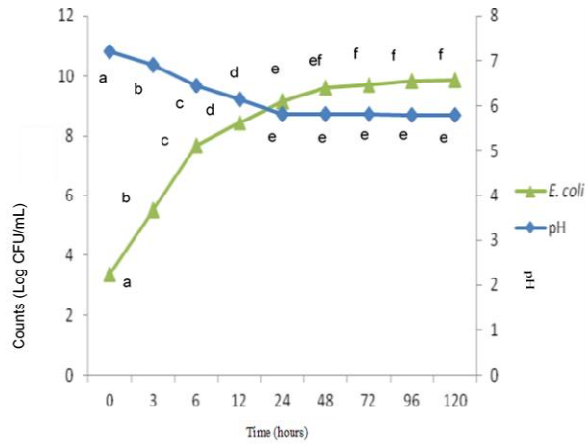


Fig. 1b. Evolution in BHI of pH and *Escherichia coli* KO 13
 Values with the same letter on a curve are not significantly different for $p>0.05$

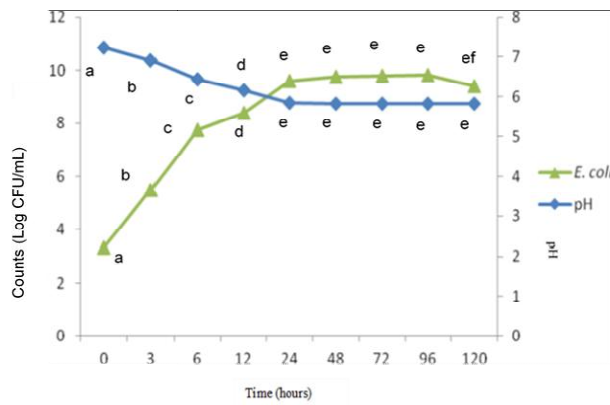


Fig. 1c. Evolution in BHI of pH and *Escherichia coli* from Tuna loins
 Values with the same letter on a curve are not significantly different for $p>0.05$

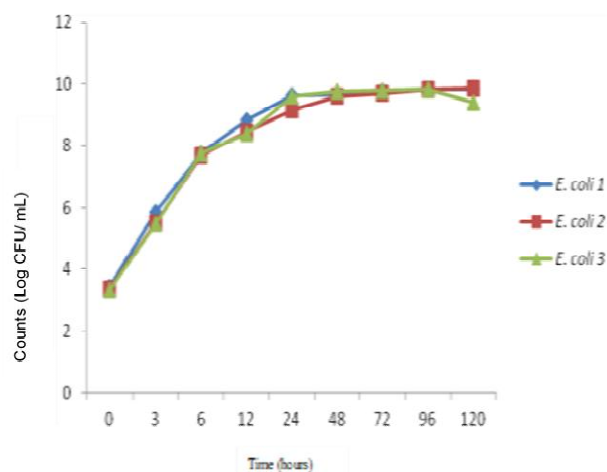


Fig. 1d. Comparative evaluation of the growth in BHI of the different strains of *Escherichia coli*
E. coli 1 = *E. coli* ATCC 25922; *E. coli 2* = *E. coli* KO 13; *E. coli 3* = pathogenic *E. coli* from Tuna loins

Table 1. Characteristic growth constants of the different *Escherichia coli* strains studied in BHI

Growth parameters	<i>E. coli 1</i>	<i>E. coli 2</i>	<i>E. coli 3</i>
CC (log UFC/mL)	6.40±0.02 ^a	6.49±0.05 ^b	6.53±0.02 ^c
μ (h ⁻¹)	1.46±0.04 ^a	1.65±0.03 ^b	1.74±0.03 ^c
G (min)	29±1.73 ^a	26±1.73 ^c	24±2.08 ^c

E. coli 1 = *E. coli* ATCC 25922; *E. coli 2* = *E. coli* KO 13; *E. coli 3* = *E. coli* from Tuna loins; CC = growth capacity; μ = specific growth rate; G = generation time; Values with different letter on a line are significantly different for $p > 0.05$

3.2 Constant Growth Characteristics of *Escherichia coli* in Liquid Medium

Table 1 presents the constant growth characteristics of the three strains of *E. coli* studied. The growth capacity (CC) values of three *E. coli* strains are close to 1, which is the reference value. The specific growth rate and generation time obtained with *E. coli* strains isolated from Tuna loins were $\mu = 1.74 \text{ h}^{-1}$ and $G = 24 \text{ min}$, respectively.

3.3 Monitoring the Growth of *Escherichia coli* in Tuna Loins

Figs. 2a to 2c illustrate the tracking of growth in Tuna loins of strains of *E. coli* ATCC 25922, *E. coli* KO 13 and pathogenic *E. coli* isolated from Tuna loins, respectively. The pH decreased from 6.05 to 5.17; 6.03 to 5.17 and 6.05 to 5.16 in the Tuna loins inoculated with *E. coli* ATCC 25922, *E. coli* KO 13 and *E. coli* strains isolated from Tuna loins respectively. Bacterial loads increased over time from 3.49 to 9.85 log CFU/g

(Fig. 2a); from 3.36 to 9.85 log CFU/g (Fig. 2b); and from 3.32 to 9.85 log CFU/g (Fig. 2c). The curves showing the evolution of the bacteria are characterized by two phases: an exponential phase from 0 to 24 h and a stationary phase from 24 h.

Fig. 2d shows the evolution of the three strains of *Escherichia coli* studied. The three curves have the same paces. They are characterized by two phases that present the physiological characteristics of the strains studied: an exponential phase reflecting rapid growth from 0 to 24 h and a stationary phase from 24 h onwards.

3.4 Characteristic Growth Characteristics of *Escherichia coli* in Tuna Loins

Table 2 shows the growth characteristic constants of the three different strains of *E. coli* studied. The growth capacity (CC) values for the three *E. coli* strains are close to 1, which is the

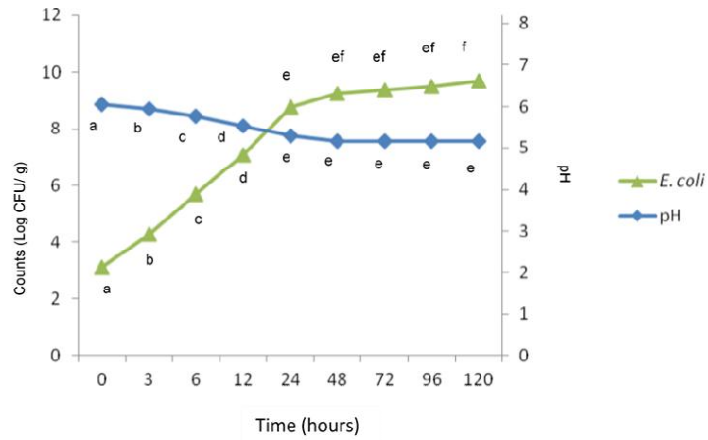


Fig. 2a. Evolution in tuna loins of pH and *Escherichia coli* ATCC 25922
 Values with the same letter on a curve are not significantly different for $p>0.05$

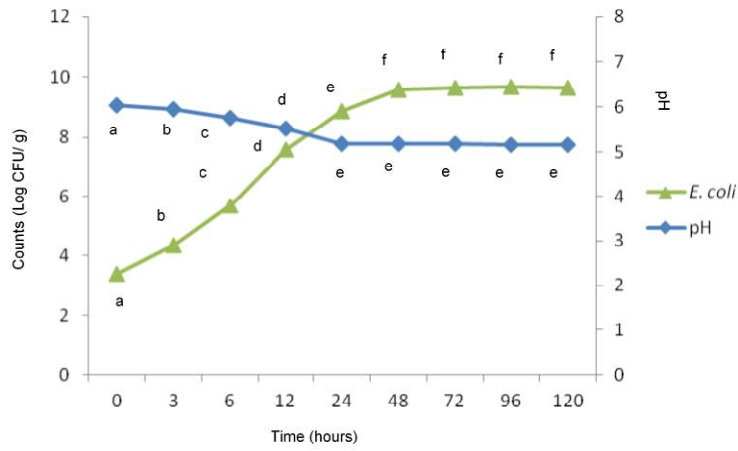


Fig. 2b. Evolution in tuna loins of pH and *Escherichia coli* KO 13
 Values with the same letter on a curve are not significantly different for $p>0.05$

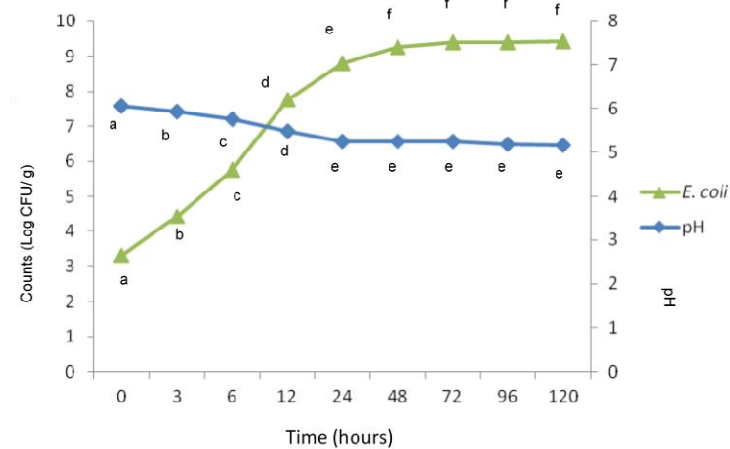


Fig. 2c. Evolution in tuna loins of pH and *Escherichia coli* from Tuna loins
 Values with the same letter on a curve are not significantly different for $p>0.05$

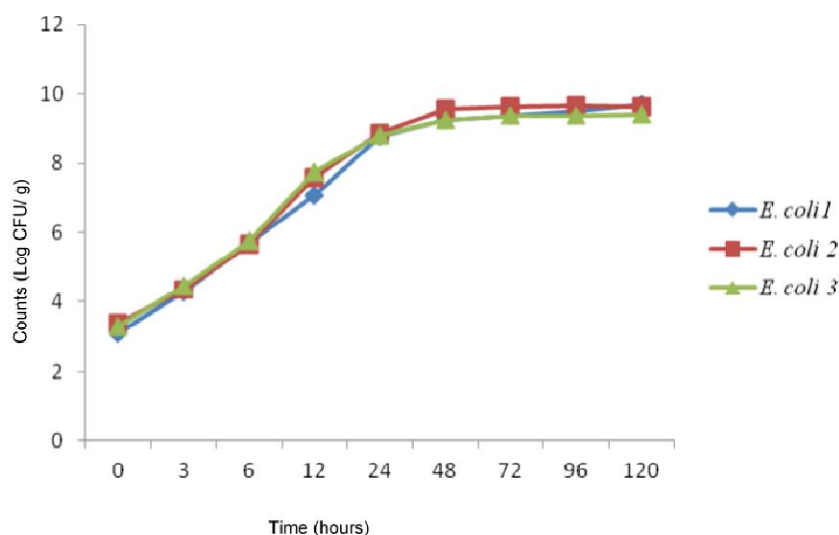


Fig. 2d. Comparative evaluation of the growth in Tuna loins of the different strains of *Escherichia coli* studied

E. coli 1 = *E. coli* ATCC 25922; *E. coli 2* = *E. coli* KO 13; *E. coli 3* = *E. coli* from Tuna loins

Table 2. Characteristic growth constants of the different *Escherichia coli* strains studied in Tuna loins

Growth parameters	<i>E. coli 1</i>	<i>E. coli 2</i>	<i>E. coli 3</i>
CC (log UFC/g)	6.24±0.07 ^a	6.29±0.05 ^b	6.28±0.08 ^b
μ (h ⁻¹)	1.06±0.02 ^a	1±0.01 ^b	1.01±0.02 ^b
G (min)	39±1.66 ^a	42±1.71 ^b	41±1.68 ^b

E. coli 1 = *E. coli* ATCC 25922; *E. coli 2* = *E. coli* KO 13; *E. coli 3* = *E. coli* from Tuna loins; CC= growth capacity; μ = specific growth rate; G= generation time; Values with different letter on a line are significantly different for $p>0.05$

reference value. The highest specific growth rate ($\mu = 1.06 \text{ h}^{-1}$) and the shortest generation time ($G = 39 \text{ min}$) were obtained with the *E. coli* strains ATCC 25922.

3.5 Growth of Pathogenic Strains of *Escherichia coli* Isolated from Tuna Loins Cultured in Liquid Medium and in Tuna Loins

Fig. 3 shows the evolution of the growth of pathogenic strains of *Escherichia coli* isolated from Tuna loins grown in liquid medium and in tuna loins. During the exponential growth phase, strains of *E. coli* inoculated in liquid medium show faster growth than those inoculated in Tuna loins. At $t = 6 \text{ h}$, the bacterial load was 7.74 log CFU / g for the strains of *E. coli* inoculated in liquid medium and 5.75 log CFU / g for *E. coli* strains inoculated in Tuna loins. During the stationary phase, the bacterial load of *E. coli* strains inoculated in liquid medium was always

higher as those inoculated in tuna loins. At $t = 72 \text{ h}$, the bacterial load was 9.79 log CFU / g for the strains of *E. coli* inoculated in liquid medium and 9.39 log CFU / g for *E. coli* strains inoculated in tuna loins.

4. DISCUSSION

Escherichia coli is not thought to survive for long periods outside the host intestine, so produce-associated outbreaks have widely been ascribed to recent fecal contamination. The suspected sources of produce contamination include soil amendments (manure or compost), irrigation water contaminated with cattle feces, or contaminated surface runoff [14;15]. The extent to which *E. coli*, in particular pathogenic strains, can survive in food and which factors affect this survival rate are crucial issues from a fundamental point of view. Survival of pathogenic *E. coli* has been studied under various conditions and in different environments. This bacterium is generally considered to survive very well in the

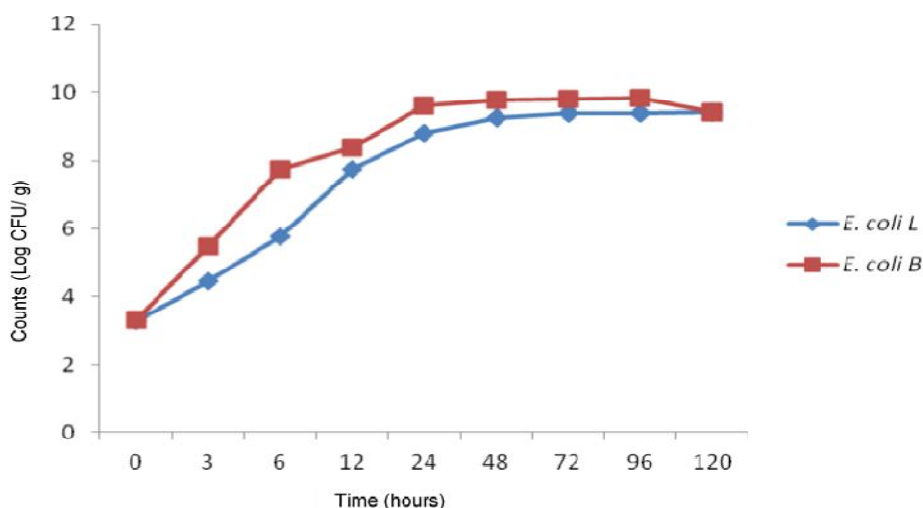


Fig. 3. Comparative evaluation of the growth of pathogenic *Escherichia coli* strains isolated from Tuna loins, grown in BHI and in Tuna loins

E. coli L = *E. coli* inoculated from Tuna loins; *E. coli B* = *E. coli* inoculated in liquid medium

environment. Its survival, however, can be impacted by various factors such as the indigenous microbial communities, temperature, dehydration, moisture and aerobic conditions [16]. *E. coli* can, to varying extents, survive in different environments such as soil, manure, water, plant and animals [17;18;19]. From infested consumable products, the organism can further spread to uncontaminated products during food processing and packaging, resulting in dissemination in the food production chain. Pathogenic *E. coli* strains thus pose a threat to the food chain and represent a still underestimated environmental risk [20].

The results of this study show that strains of *Escherichia coli* can survive in liquid media and in Tuna loins after 120 hours. These results could be explained that brain-heart broth and Tuna loins are favorable environments for the growth of *Escherichia coli*. The survival and growth of pathogenic *E. coli* in fish or processed fish products have been poorly documented. However, several studies have shown that pathogenic *E. coli* cells can survive very well for 10 to 14 days on lettuce leaves [21;22]. On various fruits, pathogenic *E. coli* is capable of survival and growth. For example, approximately 2 to 3 log of growth was produced after two days of incubation on peaches at either 20 or 25°C [23]. At lower temperatures, there was little or no growth, but survival took place for at least 14 days. Very similar results were found with mangos and papayas, in which growth and survival of pathogenic *E. coli* was observed [24].

Survival on refrigerated mangos, apples, papayas were observed for at least one month, whereas survival on cut and frozen fruits was seen for at least 180 days [24;25]. Survival of up to 21 days has been observed in untreated ground beef patties, with little decline in numbers [26]. Several authors such as [27;28;29] showed also that *E. coli* was able to survive respectively after 42 days in cheese in France; 56 days in "Maori" in New Zealand and 15 days in eggplant salads in Greece.

In this study, the three curves of *E. coli* tested have the same paces. These results are consistent with those of [30] who showed that the growth of three different strains of *E. coli* (ATCC 43895; ATCC 43890 and ATCC 43889) was the same in beef carcasses in the USA.

The characteristic growth constants of the different *Escherichia coli* strains studied in BHI employed in this study displayed faster kinetic values than those observed in Tuna loins. This could be the result of varying interactions that occur between the substrate and the pathogen that do not necessarily occur in a growing medium. According to [31], the duration of the latency phase depends on a wide variety of factors. These are the initial concentration of the inoculum and the time required to recover from physical damage or shock from the transfer. In addition, the time required for the synthesis of coenzymes or essential dividing factors and other enzymes involved in the metabolism of the substrate present in the medium.

The results of this study showed that the *E. coli* population in the liquid environment was growing faster than that in the tuna loins (solid environment). These results could be explained by the difference in the composition of the different media. The availability of nutrients such as carbon, nitrogen and phosphorus is also an important factor influencing *E. coli* survival and growth. Indeed, BHI is a liquid medium with a known chemical composition specially designed for the growth of bacterial strains, whereas tuna loins are a solid medium with a complex composition. In all natural habitats, *E. coli* populations will interact, in a loose or intricate way, with the local biota, including the microbial communities. In general, the micro-organisms develop more favorably in liquid medium than in solid medium. Authors such as [32] have shown that the type of matrices used for their seeding influenced the growth of *E. coli*. The results of this work are similar to those of [33] who showed that the growth of *E. coli* was faster in a liquid medium (Tryptone Soya Broth) than in a trout gut suspension in Scotland.

5. CONCLUSION

The objective of this work was to follow the evolution of the growth of pathogenic strains of *Escherichia coli* isolated from Tuna loins produced in Ivory Coast. The three strains of *E. coli* used in this study survived in liquid medium and in Tuna loins even after 5 days (120 hours). The growth curves of these strains all had the same appearance in liquid medium as in tuna loins. The curves showing the evolution of the bacteria are characterized by two phases: an exponential phase from 0 to 24 h and a stationary phase from 24 h. However, the growth rate of strains of *E. coli* inoculated in liquid media (BHI) was higher than that of strains inoculated in tuna loins. Pathogenic *E. coli* isolated from Tuna loins are a hazard to be considered in the microbiological risk assessment of the consumption of these Tuna products. It is therefore imperative to ensure that the Tuna loins submitted for export are of the highest bacteriological quality.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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