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Growth of enteropathogenic *Escherichia coli* strains in porridge made from an industrial flour and traditional flours of millet (*Pennicetum glaucum*) and maize (*Zea mays*)

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ABSTRACT

The growth of microorganisms in food is a major factor in the occurrence of infection. The objective of this study was to determine the growth of enteropathogenic *Escherichia coli* (EPEC) strains under room temperature in Côte d'Ivoire (25°C-35°C). In this study, seven strains of EPEC which included a strain as control (*E. coli* control), 2 strains from human (*E. coli* He33 and *E. coli* He80) and 4 strains of food origin (*E. coli* ABmi027, *E. coli* YOma031, *E. coli* Bg31, *E. coli* Pb26) where introduced into a heart infusion for 8 h. A first selection to determine the best strains allowed for the retention of 5 of them (*E. coli* He33, *E. coli* He80, *E. coli* ABmi027, *E. coli* YOma031 and *E. coli* Bg31). These ones where used to study the growth in infant porridge made from traditional maize or millet flour and industrial cereal mixed flour. Measurement of the growth of the strains was done at 25, 30 and 35°C. The best temperature for the growth of all strains was 37°C. However, 35 and 30°C are important temperatures for the growth of all strains. EPEC strains of food origin grew better than EPEC strains of human origin. Porridge from industrial mixed flour was the matrice in which the strains grew better. There were no significant differences in growth regardless of the strain, however, the nature of the porridge, the origin of the strain and temperature are key factors in the adaptation and growth of strains. The results obtained show that temperatures used for cooking infant porridge in Côte d'Ivoire promote the growth of EPEC.

INTRODUCTION

At the time of weanling, the mother milk is substituted by flour which is eaten as porridges (Tou, 2006), so infant foods must be cooked with attention particularly at time at feeding him. Industrial flours based on cereal used as complementary foods are those recommended by the international authorities in rural and urban areas for their nutritional and sanitary qualities. However, the alternative for some developing countries (DCs) is the formulation of

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foods or complementary foods made from simple, fermented or enriched cereal flours made traditionally. The making of porridges with flour from locals cereals is alternately use more in Africa such as Senegal (Tou, 2006), Burkina Faso (Traoré et al., 2003), Congo (Elenga et al., 2009), Côte d'Ivoire (Soro-Yao et al., 2014), South Africa (Kunene et al., 1999) and in another countries (Trèche, 2002; FAO, 2007; Nout, 2009). Ambient temperature is one of major factor to promote the growth of bacteria in Africa tropical areas generally and particularly in Côte d'Ivoire, where temperature varies from 30°C to 37°C. Many studies are showed the effect of temperatures on bacteria by controlling their effects on the growth of strains in food (Jay et al., 2005; Adams and Moss, 2008) also according to several authors, temperature could be an approach for the improvement and safety of food these traditional infant porridges (Mensah et al., 1991; Kouamé et al., 2016).

Most strains of *E. coli* are not a serious health hazard but serotypes such as enteropathogenic *Escherichia coli* (EPEC) can cause food poisoning. It is widely studied in humans because of its involvement in persistent and severe childhood diarrhea in developing countries and that it is the cause of death of hundreds of thousands of children per year (Chen and Frankel, 2005) in children less than 12 months and mainly among those less than five (5) (Donnenberg, 2005). Present in the environment, it affects mainly poor people with hygiene in deficit (Fagundes-Neto and Scaletsky, 2000). EPEC although rare are nevertheless present in food. Thus, in Côte d'Ivoire Dadié et al. (2014) were isolated from unpasteurized milk, South Africa in cattle (Charimba, 2004) and in France (Boutin et al., 1997). *E. coli* bacteria are health indicators in food production. Their presence in cooked foods results of re-infection because these bacteria do not usually survive during the preparation process. The main reasons for the presence of *E. coli* in foods cooked are significant non-respect and non-compliance of personal and food hygiene.

Food cooked in households and street foods (FAO, 2007) are the foods at risk. The analysis of the raw material and the manufacturing process shows that the presence of pathogens is due to a combination of contamination from utensils, hands, infant porridge themselves (Kunene et al., 1999) and additives (Ölmez and Aran, 2004). Contaminations of cooked porridge are the effect of handling, storage and packaging of inadequate (Kunene et al., 1999).

The ability of *E. coli* strains to grow in food is a favorable circumstance to the explosion of human infections associated with EPEC. Thus, ingestion of food contaminated with a quantity of EPEC cells, can lead to infantile gastroenteritis (IGE) or foodborne infections in children according to age and health of the consumer (Schmid-Hempel and Frank, 2007; Greig et al., 2010).

In domestic food cooking, temperature is the most applied method. Whether high or low, it has an impact on bacterial growth. However certain temperatures favor its growth and remain dangerous for the food and consumer. *E. coli* is a mesophilic organism, its growth various according the medium culture. But several studies have shown that strains of *E. coli* surveyed grew from at least 10 to 45°C, with some strains growing at 8°C or 7.5°C (Samuel et al., 1994; Jae-Ik et al., 2019).

In order to limit effect of bacteria such as EPEC and its consequences it should be necessary to know its behavior when it contaminated infant food at ambient temperature.

MATERIALS AND METHODS

Matrices and their preparation procedure

Food

Food samples used in the present study were made with industrial flour, traditional flour of maize and millet. Industrial flour is used because it is recommended by the international authorities. Traditional flour of maize and millet were used because of common use in the preparation of porridge at rural and urban households (Trèche and Massamba, 1991).

Two models of porridges were prepared to show the evolution of *E. coli* strains. Indeed, flour, water for cooking and all materials for the experiment were sterilized in autoclaving at 121°C for 15 min to make sure their hygienic quality (Clavero and Beuchat, 1996). The experiments were performed in aseptic conditions.

Approximately 500 g of each flour was divided into 20 portions (25 g for each), placed in sterile jars. In aseptically 260 ml of water was heated at 100°C and flour was putted into the heating water. It was stirring regularly until cooked (for 10 to 15 min). A quantity of 14 grams of sugar was added to each porridge according to traditional cooking.

Strains and preparation of *E. coli* starter (inoculum)

EPEC strains detected in a previous study were used as control strains in this study (Table 1). The cultures were activated by plating on RAPID' *E. coli* 2 (Bio-Rad, France) agar. A pure colony was inoculated in 25 ml of Brain and Heart Infusion (BHI) (Biokar) for 2 h ± 20 min at 37°C. After 2 h, 1 ml of BHI is taken every 5 min, boiled in a bain-marie at 55°C during 30-40 min and then plated on RAPID' *E. coli* 2 (Bio-Rad, France). This technique allowed obtaining the initial load of 10^2 CFU/ml. This estimated colony is dispersed in 1 ml of peptone water and used to inoculate the different matrixes.

Table 1. EPEC strain used in this study.

	Pathotype	F V	References
Pb26	EPEC	*eae, bfp, AL*	Dadié et al. (2010)
Bg31	EPEC	*eae, bfp, AL*	
He33	EPEC	*eaeA*	Dadié et al. (2014)
He80		*eaeA-bfpA, AL*	
ABmi020	EPEC	*eaeA*	Kouamé et al. (2016)
YOma031		*eaeA-bfpA*	

Pb26: EPEC strain isolated from food at Port-bouët; **Bg31:** EPEC strain isolated from food at Bingerville; **He33:** EPEC isolated from human diarrheic stool; **ABmi027:** EPEC isolated from millet porridge; **YOma031:** EPEC isolated from maize porridge; ***Eae*****:** *E. coli* attaching-effacing factor; ***Bfp*****:** bundle-forming pilus; **AL:** localized adherence.

Procedure of broth and different matrixes inoculation and enumeration

Each food sample was inoculated with 25 ml of each inoculum (10^2 UFC/ml in 25 ml). The inoculum was inoculated in porridges and maintained at temperatures of 25, 30 and 35°C. At each 1 h during 8 h, a sample has been done for realizing inoculation. At the same time, 25 g of porridges samples were taken and mixed in 225 ml of sterile peptone water (Difco) at 0.1% (Clavero and Beuchat, 1996) in a polyethylene bag and mixed with a stomacher for 2 min. One ml of sample was taken and added to 9 ml of Peptone saline diluent. Samples were serially diluted with sterile 0.1% peptone water and surface plated with 0.1 ml in duplicate on RAPID' *E. coli* 2 (Bio-Rad, France). However, for broth 1 ml was streaked on the same medium.

The same experiment was done at 37°C with broth and matrixes. For all tests three independent assays were performed for each analyzed. And also, a non-inoculated control was included for each test. The plates were incubated at 42°C, and colonies were counted after 21 ± 3 h. The same procedure was used for the control samples.

Statistical analysis

Different variables, including the number of *E. coli* were compared using Chi-carré test and the test precision (accuracy) of Fischer (Armitage and Berry, 1987). A value of $p < 0.05$ was considered as indicating a significant difference.

RESULTS

The different Figures show that the various strains of *E. coli* evolve well in the broth (BHI) at 37°C. At this temperature, we note a lag-phase of 1 h for all strains. After this phase all the strains grow very well, whether control or EPEC strains, while control strain grows better than EPEC strains. However, Human EPECs (*E. coli* He80 and *E. coli* He33) grow less than all of them. The evolution varies of 5 units during 8 h (Figure 1). The culture in the BHI was done to select the best strain to continue the experiment. The control strain and *E. coli* Pb26 were not use for the follow study.

At 25°C, we note that, the lag-phase last 2 h at least in both industrial, maize and millet porridges. In Industrial porridge, all the strain have almost the same behavior. The growth has begun at 2 to 3.4 log CFU/ml even if at 4 to 7 h there is some difficulty for human strains (Figure 2). On the other hand, in the millet and maize porridges, foods EPEC grow better than humans EPEC strains even if the growth is not significant ($p = 0.14$). According to Figure 3, *E. coli* He33 grows slowly than other strains. Concerning maize porridge, all the strains grow until 5 h (2 – 2.3 log CFU/ml) but after 5 h those are foods strains which grow better than humans EPEC (Figure 4). Whether in porridge made from industrial flour, porridge made from millet and maize, the strain *E. coli* Bg31 remains the strain which has the best growth and *E. coli* He33 the last in both millet and maize porridges. At 25°C, the growth is better in porridge made from industrial flour but most of the strain have the same values (Figures 2, 3 and 4).

At 30°C, the lag-phase is reduced in all the porridges; it is less than 2 h. The growth is visible after 3 h in the porridge from industrial flour. In this porridge, foods and humans strains grow to 2 – 2.3 log CFU/ml before 3 h. After 3 h, all the strains have a good growth until 8 h (2.3 – 4.3 log UFC/ml) (Figure 5). In the porridge from millet flour, all the strains have a good growth while, food strain *E. coli* Bg31 grows better and human strain *E. coli* He80 has low growth (Figure 6). Conversely in the porridge from millet flour where the human strain *E. coli* He33 has the low growth, in porridge from maize flour this strain was grown fast (Figure 7). According to Figures 5, 6 and 7 generally food strains have the best growth but

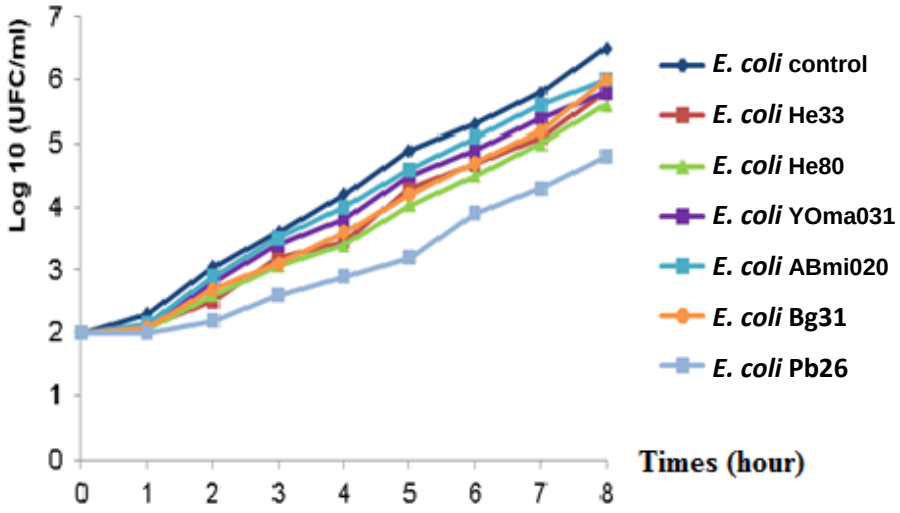

Figure 1. Growth of EPEC and *E. coli* control strain in BHI at 37°C.

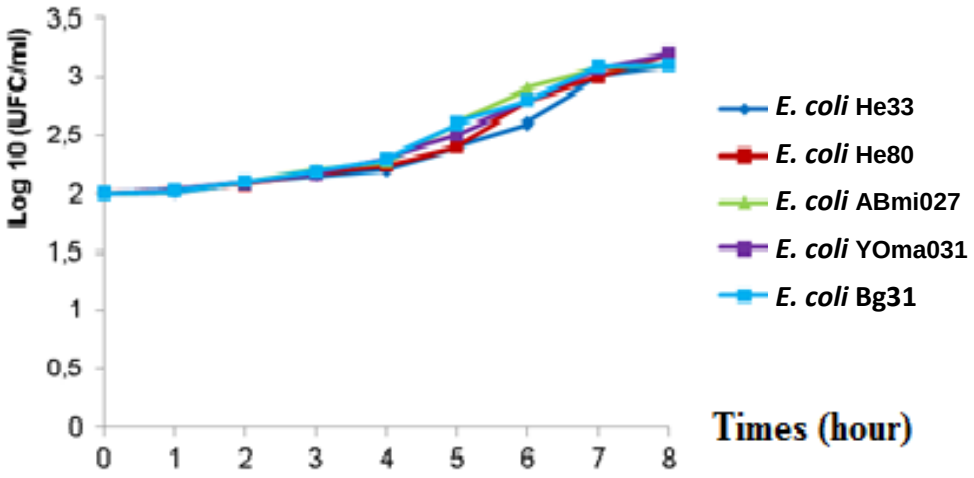

Figure 2. Growth of EPEC in porridge from industrial flour at 25°C.

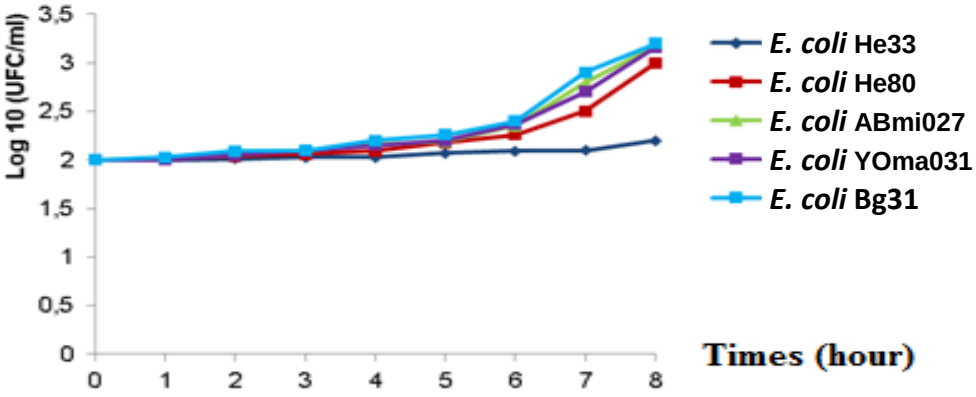

Figure 3. Growth of EPEC in porridge from millet flour at 25°C.

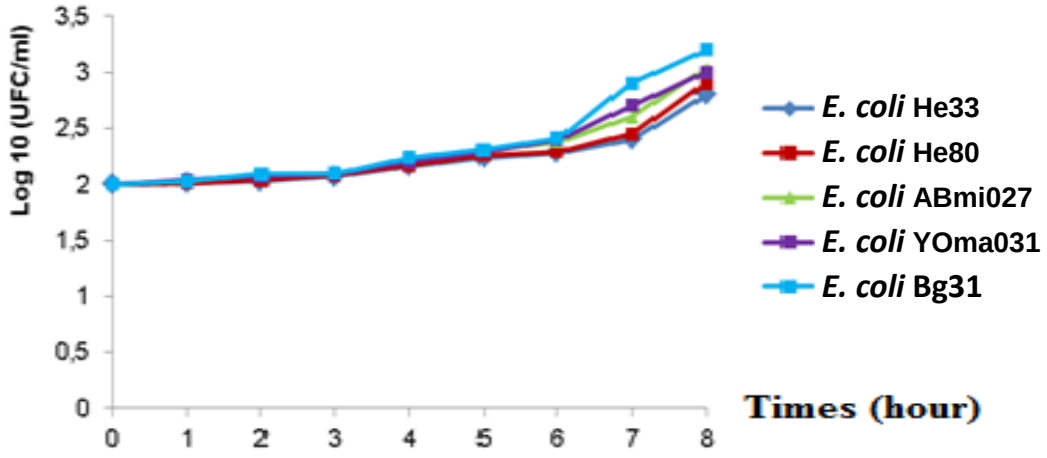

Figure 4. Growth of EPEC in porridge from maize flour at 25°C.

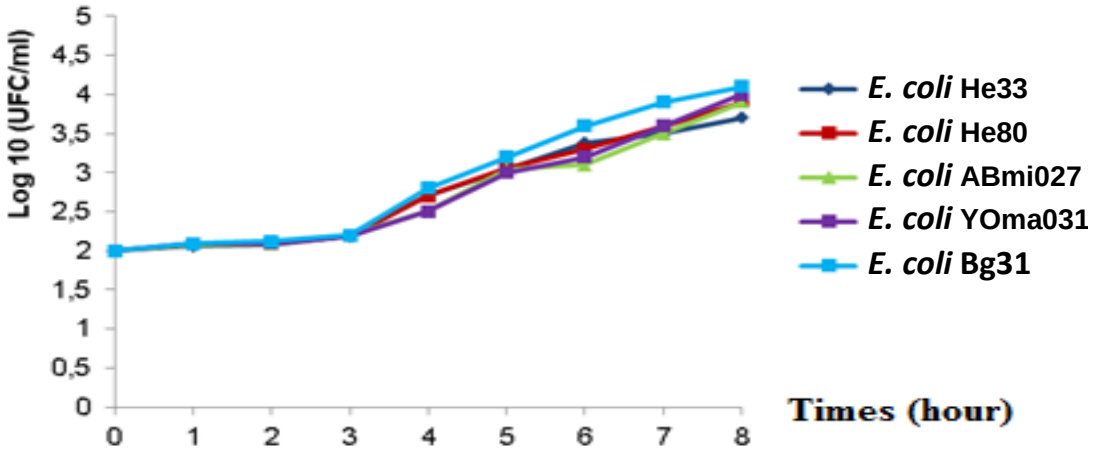

Figure 5. Growth of EPEC in porridge from industrial flour at 30°C.

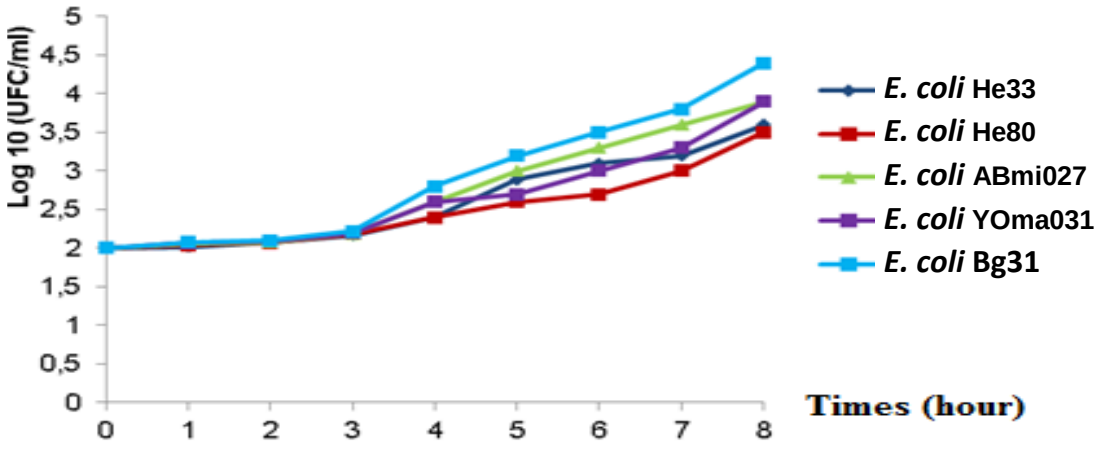

Figure 6. Growth of EPEC in porridge from millet flour at 30°C.

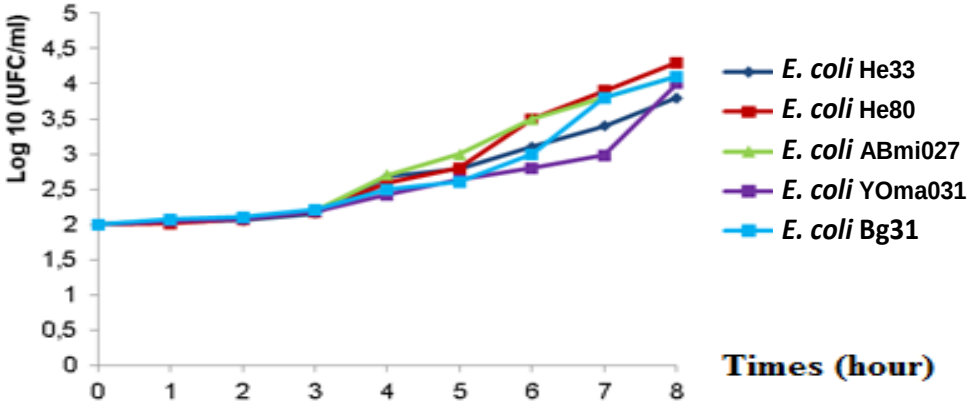

Figure 7. Growth of EPEC in porridge from maize flour at 30°C.

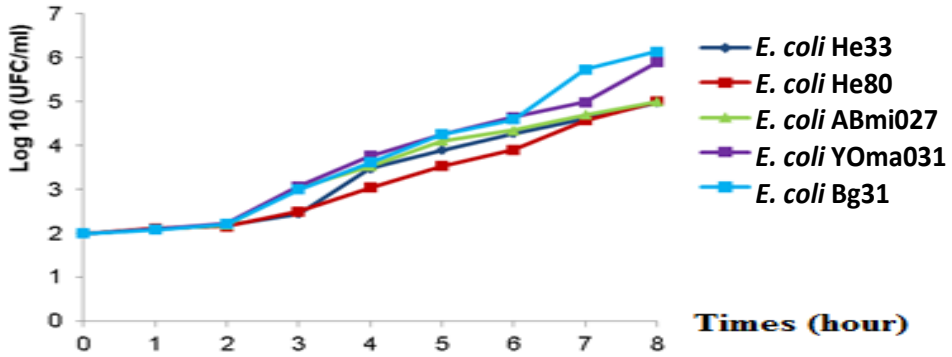

Figure 8. Growth of EPEC in porridge from industrial flour at 35°C.

especially it was *E. coli* Bg31 which has the best growth in porridge from industrial and millet flour except porridge from maize flour which strains *E. coli* ABmi027 and *E. coli* He80 during 8 h.

The growth is more appreciable at 35°C in the porridge from industrial, millet and maize flours. Conversely, in the others temperatures, at 35°C the growth of the strain have begun at 2 h. With the exception of foods strains *E. coli* Bg31 and *E. coli* Yoma31 for which the growth increases from 2.5 to 6.5 log UFC/ml during the 8 h, the other strains have a growth lower than 5.5 log in the porridge from industrial flour (Figure 8). In porridge from millet flour, those are foods strains *E. coli* Bg31 (2.2 – 4.9 log UFC/ml) and *E. coli* YOma031 (2.2 – 4.6 log UFC/ml) which have the best growth. The food strain *E. coli* ABmi027 has the low growth (Figure 9). Following the Figure 10, tree strains, *E. coli* He33 (2.1 – 4.8 log UFC/ml) followed by *E. coli* Bg31 (2.1 – 4.7 log UFC/ml) and *E. coli* Yoma31 (2.1 – 4 log UFC/ml) have the best growth while stains *E. coli* ABmi027 (2.1 – 3 log UFC/ml) and *E. coli* He33 (2.1 – 3 log UFC/ml) have the low growth (Figure 10). At the end of experiment at 35°C, the following curves show almost the same values but the behaviors of the strains change (Figures 8, 9 and 10).

DISCUSSION

In this study, the growth of the different EPECs varies with temperature. The analysis of our results shows that the growth of EPEC is greater at the temperature of 35°C in the different porridges. However, at the temperature of 25°C the growth is less important for all the strains. In addition, from temperature of 25 to 35°C EPEC strains evolve logarithmically.

Effect of temperature on the growth of EPEC strains

Enteropathogenic *E. coli* is a mesophilic germ with a growth characterized by a minimum growth temperature, an optimum growth temperature, and a maximum growth temperature. At 37°C, it proliferates anarchically in food when all conditions are met (Guiraud, 1998).

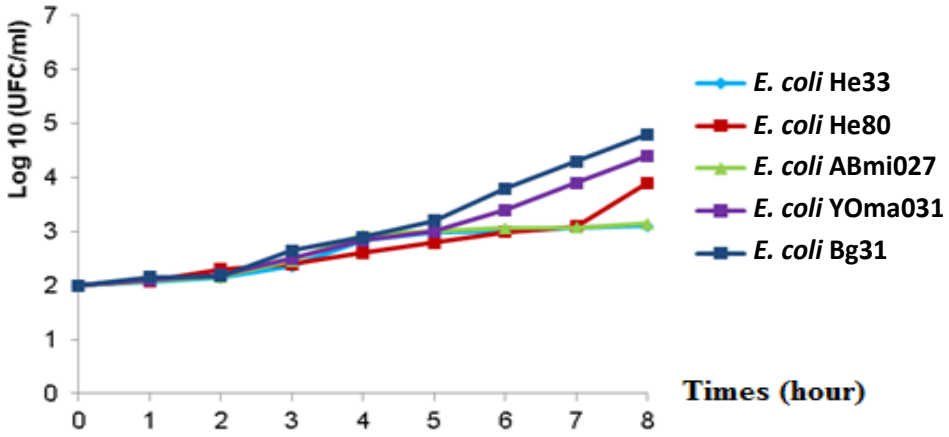

Figure 9. Growth of EPEC in porridge from millet flour at 35°C.

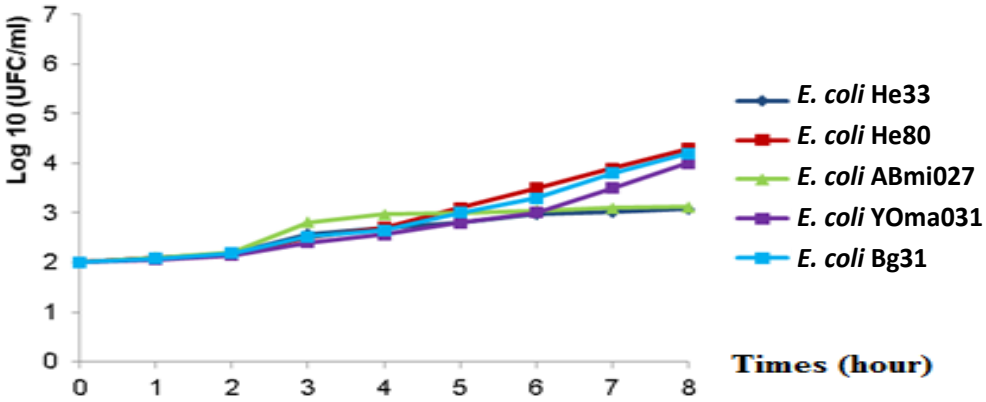

Figure 10. Growth of EPEC in porridge from maize flour at 35°C.

The results of our studies are consistent with results found by Yang and Chou (2000) who showed that the maximum growth temperature is 37°C. In addition, studies by Cooper et al. (2001) showed that the growth temperature increases linearly by 20°C and reaches the maximum after 35°C. Also, Taulo et al. (2009) showed in their study that temperatures of less than 30°C were significantly favorable for the isolation of *E. coli* and *Staphylococcus aureus* strains, followed by temperatures between 30 and 40°C. *E. coli* cells will grow over a temperature range of about 40°C, and remarkably, the cell growth rate increases in response to increasing temperature like a simple chemical reaction in a central normal range of its growth temperatures (20 to 37°C). The growth of EPEC at 35 and 37°C could be explained by the fact that, generally, an increase in temperature will increase enzyme activity. But if temperatures get too high, enzyme activity will diminish and the protein (the enzyme) will denature (Angela, 2013).

We note the reduction of the lag phase or adaptation phase of EPEC in the medium as the temperature increases. According to Robinson et al. (1998) the lag phase can be understood in terms of the amount of work to be done to adjust to new environmental conditions and the rate at which that work is done. It is explained the fact that the lag phase is less in porridges from industrial flour than in porridges from traditional flour. Also, the temperature influences the reduction of the lag time (Mellefont and Ross, 2003; Lidia and Erland, 2019).

In the broth, the increasing of the strain is greater than in porridges. This importance is justified by the fact that the BHI is a nutrient broth for *E. coli*. For its high nutritious and easily assimilated elements by non-demanding strains such as *E. coli* the broth is considered as a reference in this context for the evolution of EPEC.

The bacteria grow at a favorable temperature, and most of food for infant is given at room temperature. While, there is a relationship between exposure temperature and duration of exposure which are important parameters in food contamination and

transmission of gastroenteritis. Our study showed the real risk of bad preservation of infant foods at home temperatures. Indeed, the behaviors of the strain through the growth of EPEC strain must be a signal for all people who feed a child.

Growth of EPEC in different porridges

According to the matrices EPEC from humans and those from foods grow better in the porridge from industrial flour than in the porridges from traditional flour (flour of millet and maize). This could be explained by the fact that the growth of the strains depends to a large extent on their ability to assimilate the substrates available in their living environment. The nature of the substrate and the energy value play an important role in the rate of growth (Guiraud, 1998; Nguyen, 2008).

Carbon, hydrogen, nitrogen, oxygen, phosphorus, and sulfur are chemical elements required to build nearly all cellular components. In addition to these major elements, elements like iron, selenium, calcium, sodium, and several others are required to build specific structures and perform specific processes. *E. coli*, along with all other living organisms, requires environmental sources of all of these elements in order to survive. Most of these elements come from food sources, like carbohydrates, proteins and fats. According to Latham (2001), cereals are rich in nutrients and the fortification of cereal flour (industrial flour) with micronutrients (iron, folic acid, zinc or vitamin B9) increases the micronutrient content (Nguyen, 2008; CORAF/WECARD, 2012). As a result, all the essential nutrients for the growth of EPEC are found in porridge from industrial flours. Thus, EPECs would quickly use these nutrients easily assimilated by the strains than complex nutrients or complex polysaccharide (starch) contained in porridge from traditional flour.

In addition to these building blocks are growth factors (amino acids, nucleotides, fatty acids, or vitamins), which are in porridges from industrial flour. Despite *E. coli*'s ability to make its own growth factors, there is an advantage to supplying them. *E. coli* is able to grow and reproduce much faster if these compounds are provided. With the right conditions and a complete mix of growth factors, an *E. coli* population can double in size about every 20 min (Angela, 2013).

In this context our study has shown that porridges from industrial flours would constitute a favorable substrate for the development of EPECs. In addition, studies by Kovârovâ et al. (1996) by glucose supply showed better growth in the matrix at 37 and 40°C.

The disadvantage if germs contaminate porridges from industrial flour for children would be an alteration of hygienic quality and thus the risk of infection in children as reported by several authors (Gadaga et al., 2004; FAO, 2007; Islam et al., 2012). It is imperative to apply good hygiene practices because in 70% of cases, this endemic is caused by the absence of food hygiene during the weaning period (OMS, 2002).

Evolution of human strains and food strains

In this study, it was observed during the various experiments that food strains in general and particularly the strain *E. coli* Bg31 has a better growth at almost all temperatures (25 – 35°C). This could be explained by the fact that the food strains have already adapted to nutrient containing in medium. Indeed the strain *E. coli* Bg31 was isolated from unpasteurized milk and milk is an excellent culture medium for microorganisms, therefore a food containing all the nutrients that promote the growth of EPEC. On the other hand human strains must recognize the environment and nutrients which would explain their delay in growth in porridges.

The nature of the porridge (physico-chemical composition) and the origin of the strains are two important factors in the adaptation and growth of organisms in a porridge.

Conclusion

This study determined the effect of temperature on the growth EPEC. The results obtained showed that the post-cooking germ proliferation remains a problem in households especially for children. In Africa especially in Côte d'Ivoire, where the temperature is still around 30°C, it is imperative to respect the rules of hygiene to avoid the temperature risk zone characterized in this study as 25, 30 and 35°C. Thus, food for children's nutrition should not be stored at temperatures ranging 25 to 35°C without adequate sanitization. In addition, cooked food for children must undergo a normal pasteurization and avoid post-contamination.

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