

Acid, Bile and Aggregation Abilities of *Lactobacillus plantarum* Strains Isolated from Akamu a Nigerian Fermented Maize Food

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Abstract This study investigated the ability of two strains of *Lactobacillus plantarum* isolated from *akamu* a Nigerian fermented maize food to tolerate acid and bile condition. Auto-aggregation and co-aggregation with pathogens: *Escherichia coli* NCTC 11560 and *Salmonella* Enteritidis NCTC 5188 were also investigated. This was aimed at establishing preliminary probiotic potentials of these none intestinal *L. plantarum* isolates. Viability at pH 2 was significantly ($p \leq 0.05$) reduced from $\geq 8.26 \pm 0.05$ to $\leq 4.94 \pm 0.49$ Log₁₀ CFU/mL after 3 h. Subsequent incubation in 0.3% ox gall bile media after 6 h enhanced growth to 5.73 ± 0.13 and 7.93 ± 0.12 Log₁₀ CFU/mL for NGL5 and NGL7. The *L. plantarum* strains auto-aggregated but had no co-aggregation with the pathogens. After 5 h auto-aggregation at 37°C (>25%) was significantly ($p \leq 0.05$) greater than auto-aggregation at 22 - 24°C (<14%). The *L. plantarum* strains possessed abilities to survive passage through the GIT and auto-aggregated significantly at body temperature. This serves as a baseline data for further studies especially isolates that are not of intestinal origin.

Keywords: *Lactobacillus plantarum*, *akamu*, acid and bile tolerance, auto-aggregation, co-aggregation

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1. Introduction

Akamu is a lactic acid fermented cereal-based food that constitutes a major infant complementary food and also serves as a component of adult main meals in most African countries. To be able to develop this product for more than its nutritional value to that of health enhancing benefits would require the characterization of the fermenting microorganism for probiotic potentials. *Lactobacillus plantarum* is one of the main Lactic acid bacteria associated with spontaneous fermentation of *akamu* [1]. It is a versatile lactic acid bacterium, that is found in various indigenous fermented foods and is commonly associated with the human gastrointestinal tract (GIT) [2,3] and among the key species of the genera *Lactobacillus* that is being studied for their ability to confer health benefits.

In order to exert health-enhancing benefits, probiotics must possess certain potentials such as the ability to withstand passage through the GIT, adherence to mucus and/or human epithelial cells and cell lines, reduction of pathogen adhesion to surfaces through aggregation and inhibition of enteric pathogens [4,5]. The acidity of the human GIT depending on the food substrate and the intervals of feeding vary from pH 1.5 to 4.5 [6]. Bile in the human GIT is an important factor that affects bacteria cell viability and interaction with the environment as it is known to destroy the lipids and fatty acid components of

bacteria cell membrane [7]. Bile concentration is assumed to be higher in the upper part of the small intestine (jejunum). All bile depending on concentration are said to be inhibitory to most Gram-positive bacteria including the genera *Lactobacillus*. Viability at the site of action is presumed to be important although some effects may be mediated by cell components. Since the viability and activity of probiotics are needed at the lower digestive tract, it is expected that they should be resistant to acid and bile environment of the upper digestive tract [8].

Bacteria aggregation has been proposed to have both food preservation and therapeutic impact on intestinal microbiota [9]. Auto-aggregation has been suggested to be necessary for adhesion to intestinal epithelial cells [10], [11], while co-aggregation form barrier that prevent pathogenic colonization of the GIT through the creation of different microenvironment around the pathogens and enhanced antimicrobial activities at close proximity [12,13]. Aggregation phenomenon in many *Lactobacillus* strains are been investigated among others by spectrophotometric assays, in which bacteria aggregation are evaluated by the reduction of light absorbance in the bacterial suspended diluent using a spectrophotometer. Commonly used diluent include phosphate buffered saline (PBS) at different pH (pH 2-8) simulating both gastric and intestinal juice [11,14,15,16,17].

Although most studies had been with intestinal and dairy isolates [6,8,18], there is increasing interest not just in the development of food products containing beneficial

Lactobacillus strains but the characterisation of non-intestinal isolates for possible health-enhancing effects. This study, therefore, was aimed at investigating the acid and bile tolerance and the auto- and co-aggregation ability of *L. plantarum* strains: NGL5 and NGL7 isolated from *akamu*-a Nigerian fermented maize food as one of the important criteria for survival through the gastrointestinal track.

2. Materials and Methods

2.1. Microorganism and Inoculum Preparation

The two *L. plantarum* strains: NGL5 and NGL7 characterised in this study were previously isolated from *akamu* a Nigerian traditionally fermented maize food and identified using both conventional and molecular methods by [1]. The commercial probiotic strain (LpTx) was isolated from a probiotic food supplement obtained from Health Food Shop, Rickard Lanes', Plymouth City Centre, UK, using the same method described by Obinna-Echem *et al.*, [1]. The pathogens: *Escherichia coli* NCTC 11560 and *Salmonella enterica* serovar Enteritidis NCTC 5188 obtained from stock cultures in the microbiological laboratory of Plymouth University, UK were the pathogens used in the aggregation assay.

The lactic acid bacteria (LAB) were cultivated on de Man, Rogosa and Sharpe (MRS) agar and the pathogens on Nutrient agar incubated at 37°C for 24 h. MRS and Nutrient broths were used for the LAB and pathogens broth cultures respectively. A distinct colony from the agar plate culture was inoculated into 10 mL of broth and incubated at 37°C without agitation for 18 – 20 h. The cultures were harvested by centrifugation (Hettich Zentrifugen Rotina 46 S, Tuttlingen, Germany) at 4000 x g for 10 min and washed twice in phosphate buffered saline (PBS) (pH 7.3±0.2) and re-suspended in PBS such that 1 mL of inoculum produced 9 and 8 Log₁₀ CFU/mL for the Lab and the pathogens respectively. The media and the diluent used were obtained from Oxoid Limited (Basingstoke, Hampshire, UK).

2.2. Acid Tolerance

The ability of the *L. plantarum* strains to withstand acid condition was investigated using the methods described by Lin *et al.* [5] and Ding & Shah [8]. One millilitre of washed *L. plantarum* cell suspension (c. 9 Log₁₀ CFU/mL CFU) was inoculated into 9 mL of modified MRS broth (pH 2.0 with 5 M HCl). pH 2 has been established as the appropriate pH for screening acid tolerance of probiotic LAB [5,6,19]. The samples were incubated at 37°C for 3 h under anaerobic condition using anaerobic gas jackets (2.5 L AnaeroGen AN0025A, Oxoid, Basingstoke, England). Survival was measured by plating out serial dilutions on MRS agar plates at the beginning of the incubation time and every 1 h for 3 h.

2.3. Bile Tolerance

According to the method described Lin *et al.* [6] washed acid stressed cells were re-suspended in 10 mL

MRS broth with or without 0.3% ox gall bile (Oxoid Ltd, Basingstoke, England) and incubated anaerobically at 37°C for 24 h. The viable microbial counts were determined on MRS agar after 0, 3, 6 and 24 h of exposure. The effect of the bile salt on the growth of the *L. plantarum* strains was then determined by comparing the viability in the MRS broth with those from the bile condition.

2.4. Aggregation Analysis

2.4.1. Bacterial Auto-aggregation

Aggregation abilities of the *L. plantarum* strains and the pathogens: *E. coli* NCTC 11560 and *S. Enteritidis* NCTC 5188, were evaluated spectrophotometrically using the method described by Del Re *et al.* [11]. Briefly, 4 mL of the bacterial cell suspension (A_{600nm} of 0.5) were centrifuged (HARRIER 18/80 Refrigerated, MSE, Lower Sydenham, London, UK) at 6000 x g at 4°C for 10 min and re-suspended in same volume of their culture supernatant diluents and incubated at Room (22 - 24°C) and Body (37°C) temperatures for 2, 5 and 24 h. Absorbance (A_{600}) of 1 mL of upper suspension was measured against same volume of PBS as blank. Auto-aggregation percentage was expressed as: $(1 - A_t/A_i) \times 100$, where A_t and A_i is the absorbance at the experimental time and before incubation respectively.

2.4.2. Bacterial Co-aggregation

Bacterial suspensions were prepared as in auto-aggregation. Equal volumes (2 mL) of the *L. plantarum* and the pathogen strains (1:1 v/v) were mixed and incubated at Room (22 - 24°C) and Body (37°C) temperatures without agitation. Absorbance after mixing and after 2, 5 and 24 h of incubation was taken. Percentage Co-aggregation was expressed using the equation by Kos *et al.*, [10] as:

$$\frac{[(A_p + A_i) / 2] - A_{p+i}}{(A_p + A_i) / 2} \times 100 \text{ Where: } A_p, A_i \text{ and } A_{p+i}$$

represent the absorbance of the pathogen, *L. plantarum* and mixture of pathogen and *L. plantarum* respectively.

2.5. Statistical Analysis

Data obtained were statistically analysed using Minitab (Release 16.0) Statistical Software English (Minitab Ltd. Coventry, UK). Statistical differences and relationship among variables were evaluated by analysis of variance (ANOVA) under general linear model and Tukey pairwise comparisons at 95% confidence level.

3. Results and Discussion

3.1. Acid and Bile Tolerance Ability of Nigerian Fermented maize Food - *L. plantarum* strains

In Figure 1, viable counts of both strains of *L. plantarum* at pH 2 after 3 h were reduced from $\geq 8.26 \pm 0.05$ to $\leq 4.94 \pm 0.49$ Log₁₀ CFU/mL. Subsequent incubation of the acid stressed cells in 0.3% ox gall bile media for 6 h

resulted in growth enhancement to 5.73 ± 0.13 and 7.93 ± 0.12 Log_{10} CFU/mL for NGL5 and NGL7, respectively. The viability of the acid stressed cells in MRS and MRS with 0.3% ox gall bile for NGL5 and NGL7 are presented in Figure 2 and Figure 3, respectively. There was significant ($p < 0.05$) increase in viability with increase in time.

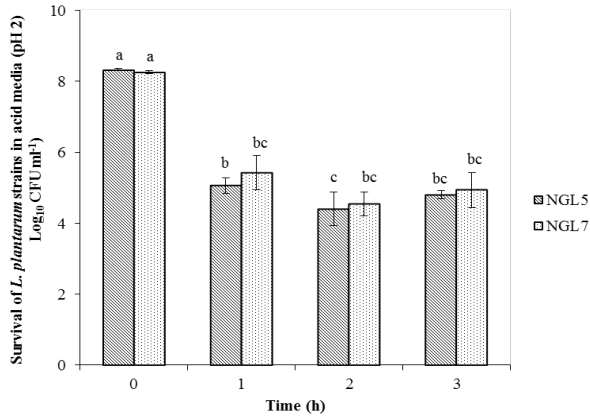


Figure 1. Effect of exposure to pH 2.0 on the viable count of two *Lactobacillus plantarum* strains (NGL5 and NGL7) isolated from Nigerian fermented maize food- akamu.

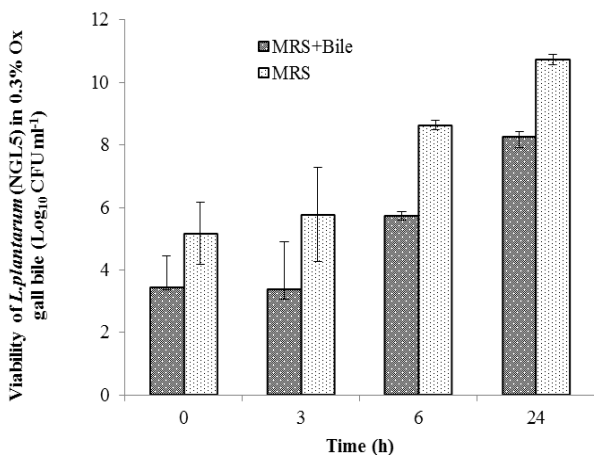


Figure 2. Viability of *Lactobacillus plantarum* (NGL5) in MRS broth with and without 0.3% Ox gall bile (NGL5 *L. plantarum* strain isolated from akamu-a Nigerian fermented maize food)

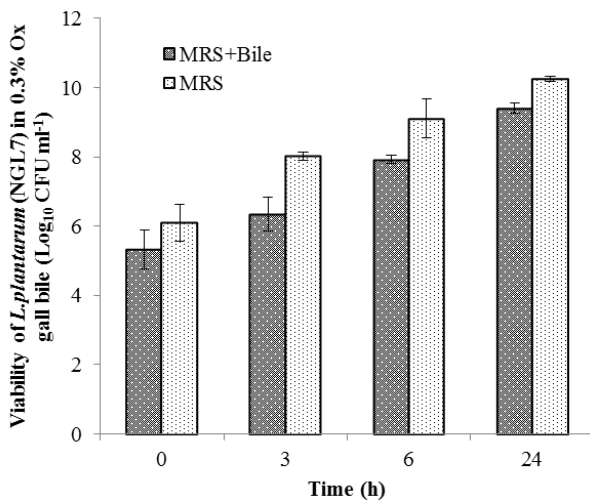


Figure 3. Viability of *Lactobacillus plantarum* (NGL7) in MRS broth with and without 0.3% Ox gall bile (NGL7 - *L. plantarum* strain isolated from akamu-a Nigerian fermented maize food)

The *L. plantarum* strains: NGL5 and NGL7 from Nigerian fermented maize study were able to withstand the acid and bile environment. Survival of approximately 5 Log_{10} CFU/mL at the end of the 3 h of exposure to pH 2 was one log cycle less than 6 Log_{10} CFU/mL reported by Ouweland & Salminen [20] as the requirement for conferment of health benefit in the host. This however, could be dependent on the initial microbial inocula and the buffering effect of the substrates in the media. The food substance in which the LAB is relayed into the GIT may influence the intensity of the adverse effect that the acid and gastric juice may have on the LAB and thereby increase the survival and viability of the organism [21]. The increase in the levels of the acid stressed *L. plantarum* cells in MRS was not significantly ($p > 0.05$) different from that of the 0.3% ox gall bile condition. This was an indication that the *L. plantarum* strains tested in this study showed significant tolerance to 0.3% ox gall bile even after exposure to acid (pH 2.0). The ability of LAB to tolerate bile salt *in-vitro* could imply their capability to survive in the human small intestine. Although, the result indicated that some cells of the NGL5 were not able to recover within the first 3 h of exposure to bile. Similar viable cell counts for *L. plantarum* strains isolated from *ikii* were reported by Kalui *et al.* [19]. Mathara *et al.* [22] reported high tolerance of *L. plantarum* strains to 0.5% ox gall after acid stress. The resistance to bile has been related to the activity of bile salt hydrolase which can hydrolyse combined bile salt and hence, reduce toxicity of the bile salt [15].

3.2. Aggregation Potential of Nigerian Fermented Maize Food *Lactobacillus Plantarum* Strains (NGL5 and NGL7)

The aggregation ability of the *L. plantarum* strains are shown in Table 1. They were able to auto-aggregate at both temperatures, although aggregation was significantly ($p \leq 0.05$) greater at 37°C than at room temperature. Auto-aggregation increased significantly ($p \leq 0.05$) with time. The auto-aggregation values obtained in this study (8 - 17% and 11 - 30% at room and body temperature respectively) were higher than the values reported for some strains of *L. fermentum* [15] but lower than that of *Bifidobacterium longum* [11]. The auto-aggregation ability of the *L. plantarum* strains would be an advantage in achieving greater mass that is necessary for enhancing tolerance to the GIT system, exerting of certain health benefits and may aid the prevention of pathogen colonization [14,23].

The *L. plantarum* strains were unable to co-aggregate with the pathogens (<6%) at both temperatures as shown in Table 2. Co-aggregation of *Lactobacillus* spp. with pathogens enhances the prevention of pathogen colonization [15], however, the *L. plantarum* strains were unable to co-aggregate with the pathogens irrespective of the experimental conditions. This was comparable with the literature report that co-aggregation of enteropathogenic *E. coli* 3014 and *S. Typhimurium* with *L. plantarum* L4 was less than 5% [10]. Although of the *L. plantarum* strains did not co-aggregated with the pathogens: *E. coli* NCTC 11560 and *S. Enteritidis* NCTC 5188, acid production by the *L. plantarum* strains during fermentation had proven to be capable of

significantly inhibiting the pathogen in the food sample [24] which is of relevance in the consumption of safe product.

Table 1. Auto-aggregation (%) of the *Lactobacillus plantarum* strains (NGL5, NGL7 & LpTx) and foodborne pathogens at two different temperatures

Bacteria	Temp. (°C)	Time (h)			
		2	5	24	
<i>L. plantarum</i> strains	LpTx	37	13.69±0.65 ^b	28.15±1.16 ^a	72.15±0.62 ^d
	NGL5	22-24	8.77±0.24 ^d	16.94±0.76 ^e	89.54±1.01 ^a
		37	17.25±0.27 ^a	29.61±0.11 ^a	61.59±0.72 ^f
	NGL7	37	11.12±0.45 ^c	24.96±0.36 ^b	67.70±1.28 ^e
		22-24	8.48±0.89 ^d	16.42±0.50 ^e	79.80±1.30 ^b
	Foodborne pathogens	<i>Escherichia coli</i> NCTC 11560	37	5.67±0.42 ^e	15.56±0.85 ^{cd}
22-24			2.91±0.51 ^f	6.36±0.84 ^e	24.23±1.17 ⁱ
<i>Salmonella</i> Enteritidis NCTC 5188		37	6.21±0.66 ^e	13.63±0.57 ^d	32.98±0.75 ^b
		22-24	2.58±0.09 ^f	6.50±1.11 ^e	34.23±1.15 ^b

Values with same superscript in the same column do not differ significantly ($p \leq 0.05$). N=3±SD

NGL5 and NGL7 – *L. plantarum* strains isolated from *akamu*-a Nigerian fermented maize food

LPTX - Commercial probiotic strain isolated from a probiotic food supplement.

Table 2. Co-aggregation (%) of the *Lactobacillus plantarum* strains (NGL5, NGL7 & LpTx) with foodborne pathogens at two different temperatures

L. plantarum	Temp. (°C)	<i>Escherichia coli</i> NCTC 11560			<i>Salmonella</i> Enteritidis NCTC 5188		
		2 h	5 h	24 h	2 h	5 h	24 h
LpTx	37	4.58±1.48 ^a	3.02±0.59 ^d	27.87±6.36 ^c	6.40±0.76 ^a	5.70±1.11 ^a	12.28±1.16 ^a
	22-24	2.29±0.70 ^{ab}	3.35±1.77 ^{ab}	1.94±6.10 ^{ab}	0.01±0.00 ^c	0.01±0.02 ^c	-0.01±0.02 ^b
NGL5	37	1.20±1.26 ^c	1.15±0.44 ^{cd}	10.67±2.23 ^b	3.43±0.23 ^b	3.21±0.29 ^b	-9.27±2.24 ^a
	22-24	2.47±0.78 ^{ab}	3.89±0.12 ^a	10.50±8.54 ^a	0.01±0.00 ^c	0.01±0.02 ^c	0.00±0.10 ^b
NGL7	37	3.41±0.21 ^{ab}	2.12±1.27 ^{ab}	11.84±2.55 ^b	2.88±0.66 ^b	1.20±0.32 ^c	11.23±1.74 ^a
	22-24	1.50±0.36 ^b	0.89±0.43 ^{bc}	1.00±1.86 ^{ab}	0.01±0.01 ^c	0.01±0.00 ^c	0.08±0.04 ^b

Values with same superscript in the same column do not differ significantly ($p \leq 0.05$). N=3±SD

NGL5 and NGL7 – *L. plantarum* strains isolated from *akamu*-a Nigerian fermented maize food

LPTX - Commercial probiotic strain isolated from a probiotic food supplement.

4. Conclusion

The study revealed that the *L. plantarum* strains were able to tolerate acid and bile condition and exhibited good auto-aggregation potentials at 37°C This suggested that the organisms would be able to form adequate mass and survive passage through the GIT.

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References

- [1] Obinna-Echem, P.C., V. Kuri, and J. Beal, *Evaluation of the microbial community, acidity and proximate composition of Akamu, a fermented maize food*. Journal of the Science of Food and Agriculture, 94(2): 331-340. 2014.
- [2] de Vries, M.C., et al., *Lactobacillus plantarum*-survival, functional and potential probiotic properties in the human intestinal tract. International Dairy Journal, 16(9): 1018-1028. 2006.
- [3] Kalui, C.M., J.M. Mathara, and P.M. Kutima, *Probiotic potential of spontaneously fermented cereal based foods - A review*. African Journal of Biotechnology, 9(17): 2490-2498. 2010.
- [4] FAO/WHO, *Guidelines for the evaluation of probiotics in food. A Joint FAO/WHO Working Group Report*. London. 2002:
- [5] Lin, W.-H., et al., *Adherent properties and macrophage activation ability of 3 strains of lactic acid bacteria*. Journal of Food Science, 76(1): M1-M7. 2011.
- [6] Lin, W.-H., et al., *Viable counts, characteristic evaluation for commercial lactic acid bacteria products*. Food Microbiology, 23(1): 74-81. 2006.
- [7] Succi, M., et al., *Bile salt and acid tolerance of Lactobacillus rhamnosus strains isolated from Parmigiano Reggiano cheese*. FEMS Microbiology Letters, 244(1): 129-137. 2005.
- [8] Ding, W.K. and N.P. Shah, *Acid, bile, and heat tolerance of free and microencapsulated probiotic bacteria*. Journal of Food Science, 72(9): M446-M450. 2007.
- [9] Collado, M.C., J. Meriluoto, and S. Salminen, *Measurement of aggregation properties between probiotics and pathogens: In vitro evaluation of different methods*. Journal of Microbiological Methods, 71(1): 71-74. 2007a
- [10] Kos, B., et al., *Adhesion and aggregation ability of probiotic strain Lactobacillus acidophilus M92*. Journal of Applied Bacteriology, 94: 981-987. 2003.
- [11] Del Re, B., et al., *Adhesion, autoaggregation and hydrophobicity of 13 strains of Bifidobacterium longum*. Letters in Applied Microbiology 31: 438-442. 2000.
- [12] Collado, M.C., et al., *Indigenous Dadih lactic acid bacteria: Cell-surface properties and interactions with pathogens*. Journal of Food Science, 72: M89-M93. 2007b.
- [13] Ouwehand, A.C., et al., *Probiotics: mechanisms and established effects*. International Dairy Journal, 9(1): 43-52. 1999.
- [14] Xie, N., T. Zhou, and B. Li, *Kefir yeasts enhance probiotic potentials of Lactobacillus paracasei H9: The positive effects of coaggregation between the two strains*. Food Research International, 45(1): 394-401. 2012.
- [15] Bao, Y., et al., *Screening of potential probiotic properties of Lactobacillus fermentum isolated from traditional dairy products*. Food Control, 21(5): 695-701. 2010.

- [16] Zhang, Y., et al., *Antimicrobial activity against Shigella sonnei and probiotic properties of wild lactobacilli from fermented food*. Microbiological Research, 167(1): 27-31. 2011.
- [17] Tuo, Y., et al., *Aggregation and adhesion properties of 22 Lactobacillus strains*. Journal of Dairy Science, 2013. 96(7): 4252-4257.
- [18] Lin, W.H., et al., *Immune effect of heat-killed multistrain of Lactobacillus acidophilus against Salmonella typhimurium invasion to mice*. Journal of Applied Microbiology, 102(1): 22-31. 2007.
- [19] Kalui, C.M., et al., *Functional characteristics of Lactobacillus plantarum and Lactobacillus rhamnosus from Ikii, a Kenyan traditional fermented maize porridge*. African Journal of Biotechnology, 8(18): 4363-4373. 2009.
- [20] Ouwehand, A.C. and S.J. Salminen, *The health effects of cultured milk products with viable and non-viable bacteria* International Dairy Journal, 8: p. 749-58. 1998.
- [21] Patel, H.M., et al., *Influence of malt, wheat, and barley extracts on the bile tolerance of selected strains of lactobacilli*. Food Microbiology, 21: p83-89. 2004.
- [22] Mathara, J.M., et al., *Functional characteristics of Lactobacillus spp. from traditional Maasai fermented milk products in Kenya*. International Journal of Food Microbiology, 126(1-2): 57-64. 2008.
- [23] Jankovic, I., et al., *Contribution of Aggregation-Promoting Factor to Maintenance of Cell Shape in Lactobacillus gasseri 4B2*. Journal of Bacteriology, 185(11): 3288-3296. 2003.
- [24] Obinna-Echem, P.C. *Inhibitory Activity of Lactobacillus plantarum Strains from Akamu - A Nigerian Fermented Maize Food against Escherichia coli*. American Journal of Food Science and Technology 3(5): 118-125. 2015.