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ANTAGONISTIC EFFECTS OF THREE LACTIC ACID BACTERIAL STRAINS ISOLATED FROM NIGERIAN INDIGENOUS FERMENTED OGI ON *E. COLI* EKT004 IN CO-CULTURE

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E. coli is one of the major pathogenic bacteria that cause diarrhoea in human. Traditional fermented foods, e.g. Ogi, has been used indigenously to treat diarrhoea. This study was aimed at investigating the antagonistic activity of selected lactic acid bacteria (LAB) isolated from three varieties of ogi against multidrug resistant *E. coli* EKT004.

Antibiotic susceptibility of the tested *E. coli* EKT004 strain to ofloxacin, gentamycin, cefuroxime, ceftazidine, lincomycin, oxacillin, cloxacillin, cefotaxime, ciprofloxacin, and nitrofurantoin was tested by disc diffusion method. *E. coli* EKT004 was co-incubated in two different experiments with *Weissella paramesenteroides*, *Lactobacillus plantarum*, and *L. fermentum* that have been previously isolated from Ogi. An 8 h old *E. coli* was introduced into an overnight culture of LAB and a fresh *E. coli* was inoculated into overnight culture of LAB. Viable count of pathogen at 0 h and after 24 h co-incubation at 37 °C was observed.

The tested *E. coli* EKT004 was resistant to cefuroxime, ceftazidime, lincomycin, oxacillin, cloxacillin, and cefotaxime. The tested LAB isolates have a broad spectrum of activity against *E. coli* EKT004 used for the study with a decrease of 6–8 log of *E. coli* as compared with the control. These results indicate a direct effect of lactic acid bacterial strains on multidrug resistant *E. coli* strain.

Keywords: co-culture, lactic acid bacteria, diarrhoea, resistance

Most gastrointestinal tract diseases caused by bacterial pathogens or their toxins are spread through food or water. Transmission follows the faecal-oral route where contaminated food or water represents the vehicle for transmission. *E. coli* is one of the organisms most frequently isolated from different clinical cases of diarrhoea (Okeke et al., 1999; Tobih et al., 2006). Diarrhoea, which is an illness characterized by an increase in frequency and fluidity of stools is one of the most common diseases causing infant death in developing countries (Cheesbrough, 1994; Walderman, 1998). Diarrheal diseases continue to be a major cause of morbidity and mortality worldwide. The cornerstone of treatment recommended by the World Health Organization (WHO) remains the use of oral rehydration solution (ORS). As emphasized by Guandalini (2002), despite dramatic progresses in the understanding of the pathophysiology of diarrhoea, the list of drugs available is indeed short. The administration of antimicrobial agents, therapeutically or prophylactically, causes disturbances in the ecological balance between the host and the normal microbiota, leading to intestinal colonization by potentially pathogenic microorganisms and overgrowth by opportunistic microorganisms already present, followed by diarrhoea and fungal infections (Sullivan et al., 2001).

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Furthermore, antimicrobial resistance has become a serious public health problem worldwide. Infections caused by resistant bacteria have been shown to be more frequently associated with increased morbidity and mortality than those caused by susceptible pathogens (Helms et al., 2002; Travers & Barza, 2002). Some studies have already shown that *E. coli* often resist antibiotics such as ampicillin, amoxicillin-clavulanic acid, cotrimoxazole, nalidixic acid, and cephalothin (Alshara, 2011). Several factors result in increasing antimicrobial drug resistance rates in poor countries such as irrational antimicrobial drug usage and conditions of poor sanitation (Okeke et al., 1999; Bartoloni et al., 2006; Tobih et al., 2006).

The addition of a medication to the WHO protocol for the treatment of acute diarrhoea in children is controversial. Recently, in addition to ORS, several new therapeutic strategies, including selected *Lactobacillus* strains and biotherapeutic agents containing selected, heat-killed *Lactobacillus* strains, have demonstrated considerable potential for promoting a more rapid recovery from acute, watery diarrhoea by children with microbial enteritis (Isolauri et al., 2002).

Ogi is an acid fermented cereal gruel or porridge made from maize (*Zea mays*) or corn: sorghum (*Sorghum vulgare*) also known as guinea corn or millet (*Pennisetum americanum*) (Ohenhen & Ikenebomeh, 2007). It is the most popular traditional health-sustaining fermented food in Western Nigeria, and serves as weaning foods for infants. In some communities in south-western Nigeria, Ogi is normally administered to people having gastroenteritis to minimize discomforts (Aderiye & Laleye, 2003; David & Famurewa, 2010). Lactic acid bacteria have been directly involved in the fermentation of Ogi, but only minimal studies have shown their antimicrobial potential against gastrointestinal pathogens. Therefore, this study was carried out to evaluate the ability of LAB isolated from Ogi to prevent the growth of *E. coli* strain in co-culture.

1. Materials and methods

1.1. Microorganisms

Lactic acid bacterial strains have been previously isolated from Ogi, a Nigerian fermented cereal gruel, and identified by partial sequencing of the 16S rRNA gene. Three of the identified LAB strains were selected for co-culture studies based on their efficient antagonistic properties. They are: *Weissella paramesenteroides* AFN004, *Lactobacillus fermentum* AFN018, and *L. plantarum* AFN021. Clinical strain of *E. coli* EKT004 isolated from gastrointestinal tract was collected from Medical Microbiology unit, Ekiti State University Teaching Hospital, Ado-Ekiti, Ekiti State Nigeria.

1.2. Antibiotic susceptibility of E. coli *EKT004*

The susceptibility of *E. coli* EKT004 to various antibiotics was tested by disc diffusion method. The antibiotics used were ofloxacin (30 µg), cefuroxime (30 µg), ceftazidine (30 µg), lincomycin (30 µg), oxacillin (10 µg), cloxacillin (10 µg), augmentin (10 mg), cefotaxime (30 µg), ciprofloxacin (30 µg), nitrofurantoin (100 µg), and gentamicin (30 µg). The susceptibility of the test organism to the used antibiotics was evident by clear zones of inhibition around the antibiotics disks, and the results were interpreted according to the guidelines of European Committee on Antimicrobial Susceptibility Testing (EUCAST, 2015).

1.3. Co-culture of lactic acid bacteria and E. coli

The interference of LAB with the growth of pathogenic strains was evaluated by co-incubating *Escherichia coli* EKT004 individually with three representative strains of LAB (*Weissella paramesenteroides* AFN004, *L. fermentum* AFN018, *L. plantarum* AFN021) according to the modified method of Drago and co-workers (1997). This was done in two series of experiments.

In the first experiment, 1 ml of *E. coli* EKT004 corresponding to 2.96×10^{11} CFU ml^{-1} was inoculated into 5 ml double strength nutrient broth, and then added to 5 ml of overnight culture of LAB and incubated for 24 h at 37 °C. The monoculture of the LAB and *E. coli* was evaluated at time zero (t_0) by plating from an appropriate dilution factor onto MRS agar and MacConkey agar, respectively, followed by incubation at 37 °C for 24 h. Then, the mixture was serially diluted, and the appropriate dilution factor was plated accordingly to determine the colony counts of LAB and pathogen.

For the second experiment, both *E. coli* and the LAB were grown for 24 h at 37 °C. From the overnight culture of the pathogen corresponding to 2.96×10^{11} CFU ml^{-1} 1 ml was inoculated into fresh nutrient broth and incubated for 8 h at 37 °C. After that the pathogen culture was centrifuged (for 10 min at 10 000 r.p.m.) and the supernatant discarded, then 5 ml of double strength nutrient broth was added to resuspend the pellet, vortexed, and added to 5 ml of overnight culture of LAB making the whole mixture 10 ml. Serial dilution of both the pathogen alone (monoculture) and the mixture (co-culture) was done at 8 h, immediately after mixing, followed by plating out at appropriate dilutions. After 24 h incubation at 37 °C, appropriate serial dilutions of the mixture (co-culture) were plated on their respective selective media. Discrete colonies were counted and recorded.

2. Results and discussion

The menace of antibiotic resistance is a worldwide threat with accompanied therapeutic failure. This study reports the resistance of tested *E. coli* strain to 60% of tested antibiotics. The pathogen was resistant to lincomycin, oxacillin, cloxacillin, and ceftazidine. Denver (2004) reported that amoxicillin and cloxacillin have no significant activity against Gram-negative pathogens and *Pseudomonas aeruginosa*. The test pathogen was also resistant to cefuroxime. Previous studies (Osterblad et al., 1999; Reda et al., 2011) have shown that some pathogenic isolates from the *Enterobacteriaceae* family (*Salmonella* spp., *Shigella* spp., and *E. coli*) are resistant to cefuroxime, ampicillin, and amoxycillin/clavulanate. These results suggest that lincomycin, oxacillin, cloxacillin, ceftazidine, and cefuroxime should not be used in treating infections caused by pathogenic *E. coli* and other related diarrhoeagenic pathogens, as these pathogens have developed resistance to these antibiotics. The tested strain was sensitive to ofloxacin, gentamycin, ciprofloxacin, and nitrofurantoin. This result is in agreement with Osterblad and co-workers (1999) and Yismaw and co-workers (2006), whose pathogenic isolates exhibited the least resistance to the above-named antibiotics.

In the present study we have shown that *W. paramesenteroides* AFN004, *L. fermentum* AFN018, and *L. plantarum* AFN021, which have been previously isolated from uncooked white Ogi (*W. paramesenteroides* AFN004, *L. plantarum* AFN021) and sorghum Ogi (*L. fermentum* AFN018), effectively inhibited the growth of *E. coli* EKT004, either when inoculated after 8 h and 24 h of growth of pathogen or when cultured overnight and then

incubated with the pathogens. In contrast, the growth of the LAB was not significantly influenced by the presence of the pathogen (Figs 1–3).

Co-culture studies of overnight culture of *W. paramesenteroides* isolated from uncooked white Ogi showed that this LAB has a broad spectrum of activity against *E. coli* used for the study with a decrease of 6–8 log of the pathogen as compared with the control (Fig. 1). Previous studies (Pal & Ramana, 2010) revealed that the purified bacteriocin from *W. paramesenteroides* isolated from cucumber exhibited a broad inhibitory spectrum against foodborne pathogens and spoilage microorganisms, including Gram-negative bacteria such as *Salmonella Typhimurium*, *Vibrio parahaemolyticus*, *Aeromonas hydrophila* and *Listeria monocytogenes*. They also noted that in spite of various bacteriocins studied worldwide, studies on bacteriocins of *Weissella paramesenteroides* remain rare, hence the need to utilize the potentials of *W. paramesenteroides* isolated from Ogi as a probiotic. This study also emphasizes on the need to consume more of uncooked Ogi because of its probiotic benefits.

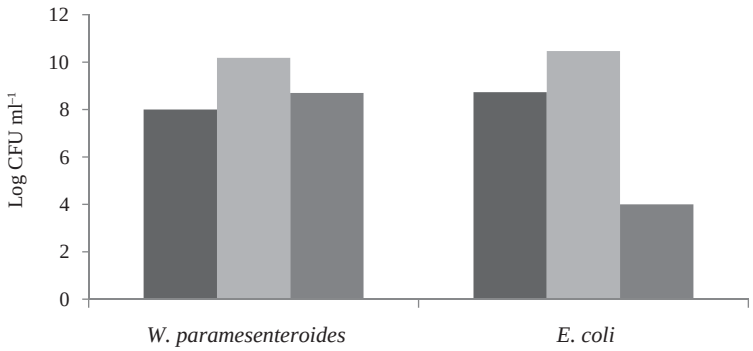

Fig. 1. Inhibition of in vitro growth of *E. coli* EKT004 by *W. paramesenteroides* AFN004 in co-culture
■: Control at 0 h; ■: control at 24 h; ■: co-culture at 24 h

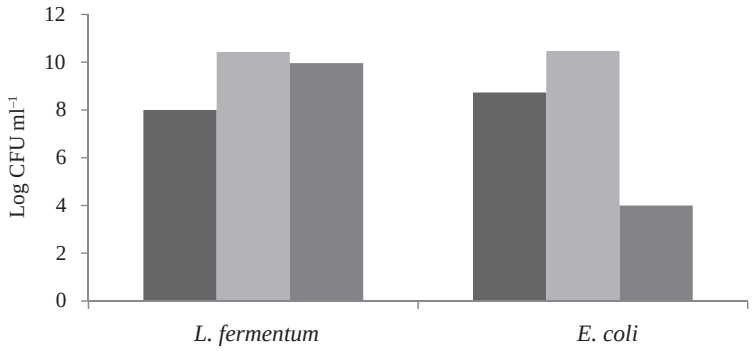

Fig. 2. Inhibition of in vitro growth of *E. coli* EKT004 by *L. fermentum* AFN018 in co-culture
■: Control at 0 h; ■: control at 24 h; ■: co-culture at 24 h

Co-culture studies of overnight culture of *L. fermentum* revealed that this LAB reduced the concentration of the cells of multidrug resistant *E. coli* by 6 log as compared to the control (Fig. 2). In the 8 h co-culture experiments, the concentration of *E. coli* cells decreased by 1 log, while at 24 h, the concentration of *E. coli* cells decreased by 5 log as compared to the control (Figs 4–6). Allaart and co-workers (2011) also reported that co-culturing of *Clostridium perfringens* with *L. fermentum* under in vitro conditions showed that, *L. fermentum* was capable of silencing beta2 toxin production of *C. perfringens* without influencing bacterial viability.

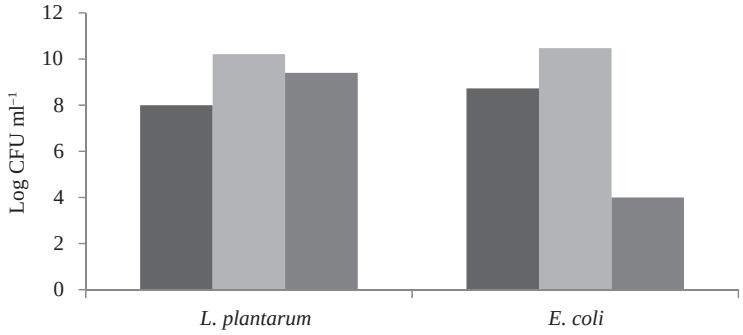

Fig. 3. Inhibition of in vitro growth of *E. coli* EKT004 by *L. plantarum* AFN021 in co-culture
■: Control at 0 h; ■: control at 24 h; ■: co-culture at 24 h

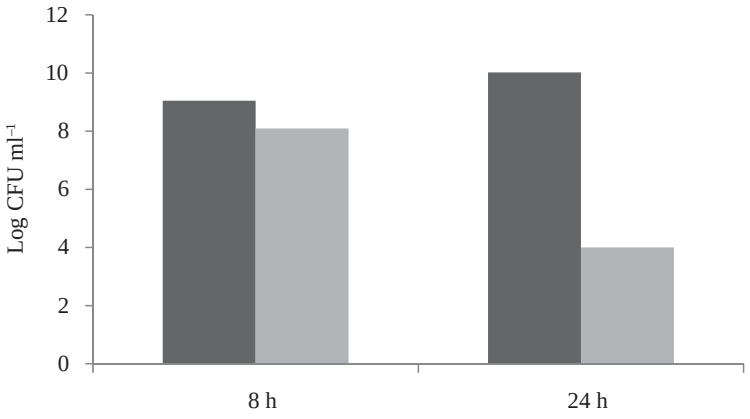

Fig. 4. Antimicrobial activities of *W. paramesenteroides* AFN004 against 8 h grown *E. coli* EKT004 in co-culture
■: Control; ■: co-cultured with *W. paramesenteroides*

This co-culture study also revealed and confirmed the antagonistic activity of *L. plantarum* against *E. coli*. There was a 6 log reduction of the concentration of the pathogen cells when co-cultured with a 24-h culture (Fig. 3). This study was in agreement with previous studies by Szala and co-workers (2012), where they reported that during two-day culture of

Salmonella Senftenberg and *Lactobacillus plantarum* strains No. 6 and 15 and *L. brevis* No. 8, total inactivation of the pathogen was observed in all the tested mixed cultures with the largest decrease (in the count of *Salmonella* Senftenberg rods in mixed culture) between the 16th and the 24th h of incubation, when the concentration of pathogen cells fell rapidly from values of 6 log to no detection level.

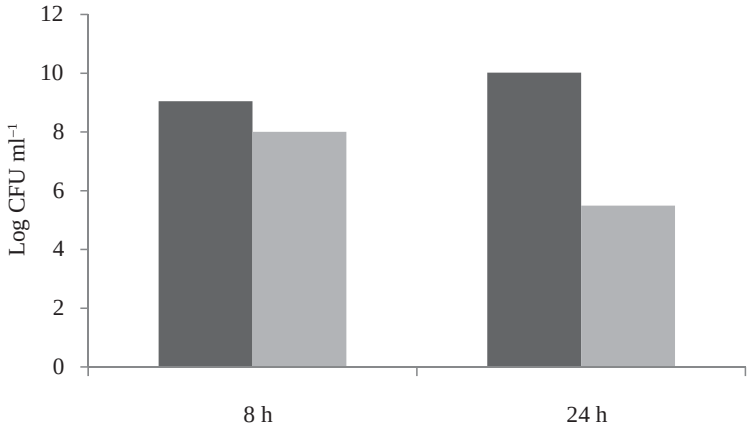

Fig. 5. Antimicrobial activities of *L. fermentum* AFN018 against 8 h grown *E. coli* EKT004 in co-culture
■: Control; ■: co-cultured with *L. fermentum*

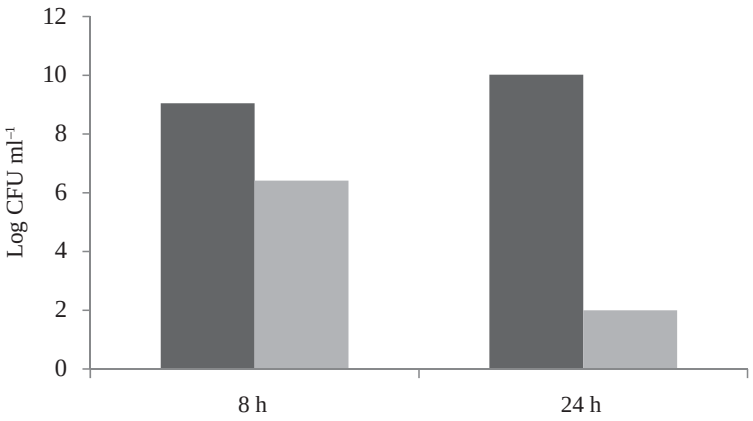

Fig. 6. Antimicrobial activities of *L. plantarum* AFN021 against 8 h grown *E. coli* EKT004 in co-culture
■: Control; ■: co-cultured with *L. plantarum*

In the second co-culture experiment, at 8 h, the concentration of *E. coli* cells decreased by 3 log, while at 24 h, the concentration of *E. coli* cells decreased by 8 log as compared to the control (Figs 2, 5). Alakomi and co-workers (2000) reported that organic acids produced by LAB are the agents that inhibited the growth of Gram-negative bacteria such as *E. coli* and *Salmonella* sp.

3. Conclusions

The presence of multidrug resistant *E. coli* strains has necessitated the search for other agents outside antibiotics that could reduce the population of the pathogen in the infectious site. This study shows that *Weissella paramesenteroides*, *Lactobacillus plantarum*, and *L. fermentum* naturally present in indigenous fermented Ogi reduced *E. coli* viable counts in co-culture and would have a great potential in inhibiting the growth of gastrointestinal *E. coli* when Ogi containing the tested LAB is consumed. This proves that the beneficial bacteria present in indigenous fermented foods have antimicrobial properties against gastrointestinal pathogens.

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