Short Communication

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IN VITRO ASSESSMENT OF THE PROBIOTIC PROPERTIES OF *LACTOBACILLUS ACIDOPHILUS* FROM FAECES AND FRESH COW MILK

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Probiotics are viable bacteria used as feed additives, which produce beneficial effects that bring about a balance of the intestinal flora. Strains of *Lactobacillus, Pediococcus, Bacteriodes, Bifido bacterium, Bacillus, Streptococcus and Escherichia coli* have been used as probiotics (Fuller 1986).

Lactobacilli are found in the normal intestinal flora of chickens and other animals from the first few days of their life (Fuller 1986). Their ability to inhibit both Gram- positive and Gram-negative bacteria had been reported (Klaenhammer 1988; DeVuyst and Vandemme 1994; Jin *et al* 1996). Chang and his co-workers (Chang *et al* 2001) recently reported that *Lactobacillus reuteri* BSA131 sourced from pig faeces strongly inhibited pathogenic bacteria used as indicator organism. Moreover, the adhesion of *Lactobacilli* to the epithelial wall of the small intestine of some animals had also been reported (Sarra *et al* 1992; Jin *et al* 1996).

Lactobacillus acidophilus produce lactic acid and small amount of hydrogen peroxide to suppress harmful bacteria (Price and Lee 1970; Gilliland and Speck 1977). Some strains produce bacteriocins such as acidophilin, lactocidin and acidolin (Gilliland 1989). The resistance of *Lactobacillus acidophilus* to bile salt and pH had been reported (Andrez and Leszek 2001).

Studies on the antagonistic effect of wild strain of *Lactobacillus acidophilus* from faeces and fresh milk on some pathogen and their adherence to the IEC (Ileum epithetial cell) of albino rat is lacking. The aim of the present work was to investigate the antagonistic effect and adhesion properties of *Lactobacillus acidophilus* strains to the IEC of albino rat.

Source of Lactobacillus isolates. Fresh cow milk was obtained from the university farm. Faeces of man was collected from a student while faeces of pig and albino rat were obtained from the piggery and experimental animal house of the Federal University of Technology, Akure, Nigeria.

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Isolation and characterisation of isolates. *Lactobacillus* isolates were obtained using MRS agar (LABM). Incubation was at 37˚C under an anaerobic environment generated inside a dessicator. Dry crab – like colonies with projecting outgrowth were subjected to morphological and biochemical test for identification (Parker and Collier 1990).

Source of indicator bacteria. Pure culture of *Bacillus cereus* NCIB 6349, *Escherichia coli* Type 1 NCIB 86, *Pseudomonas aeruginosa* NCIB 950, *Klebsiella pneumoniae* NCIB 14070, and *Staphylococcus aureus* NCIB 8588 were obtained from culture collection of the Department of Microbiology, Obafemi Awolowo University, Ile Ife, Nigeria. They were maintained on nutrient agar (LABM) slant throughout the duration of the study.

In vitro antagonism assay. The agar diffusion assay of Schillinger and Lucke (1989) was used to assay the inhibitory effect of *Lactobacillus acidophilus* strains against the indicator bacteria. This involves seeding Tryptone Soya agar (LABM) plates with the test bacteria and introducing 0.05ml (50 µl) of overnight broth culture *L.acidophilus* into holes bore with 3mm cork borer. The plates were incubated aerobically at 37˚C for 24h after which they were examined for zones of inhibition.

Preparation of intestinal epithelial cell of albino rat. Ileum epithelial cell of 4-weeks-old albino rat was prepared by using the method of Jin *et al* (1996). The epithelial cells of the ileum were scrapped off gently using the edge of a microscope slide and the scrapping suspended in phosphate buffer saline (PBS) of pH 7.3. The suspended scrapping was stored in ice for 15 min to allow the debris to settle. The sedimented debris was removed and the supernatant fluid centrifuged for 10 min at 2 g to remove large tissue cluster. It was centrifuged again at 120 g for 10 min to spin down the cells in suspension. The IEC was then suspended to a concentration of 6×10^5 cell / ml.

In vitro adhesion assay. The method described by Jin *et al* (1996) was adopted. Cells from overnight anaerobic cultures of *Lactobacillus acidophilus* in MRS broth was washed and suspended in PBS to a density of 10^8 cells/ml. One fifth of 1ml (0.2ml) of epithelial cell suspension was added to 0.8ml of the overnight anaerobic broth culture of *L.acidophilus* and incubated for 1h at 37˚C. Adhesion of the *L.acidophilus* strains to IEC was assessed using light microscopy with phase contrast illumination (x100).

Statistical analysis. The differences between the mean of the *L.acidophilus* attached to the IEC was assessed using the one way ANOVA (SPSS version 10.0) with the level of significance set at $P < 0.05$.

Table 1
Antimicrobial activities of faecal strains of *L.acidophilus* strains towards indicator bacteria

Indicator bacteria	Isolates Zone of inhibition (mm)*			
	1H	1P	1A	1C
Bacillus cereus	3.0 ± 1.0	3.5 ± 1.0	3.5 ± 1.0	NI
Escherichia coli	2.5 ± 0.0	1.0 ± 1.0	1.5 ± 0.5	6.0 ± 1.7
Pseudomonas aeruginosa	NI	2.5 ± 0.9	NI	15.0 ± 0.5
Klebsiella pneumoniae	4.0 ± 0.1	3.0 ± 1.0	2.0 ± 1.0	NI
Staphylococcus aureus	3.5 ± 1.3	2.0 ± 0.5	NI	NI

*Values are mean ± SD of three replicates; NI: No inhibition
H: Human isolate; P: Pig isolate; A: Albino rat isolate; C: Cow milk.

Table 2
Attachment of *L.acidophilus* strains to the IEC of albino rat

Isolates	Bacteria / IEC *
1H	15
1P	5
1A	23**
1C	10

*Each value is a mean of three replicates. **Value is higher and significantly different ($P < 0.05$) from other. Contact time (1h) at 37°C.

L. acidophilus strain was isolated from fresh cow milk, human, pig and albino rat faeces, respectively. The strain from pig was able to inhibit all the indicator bacteria (Plate B) while the isolate from the other sources could not inhibit one or two of these bacteria (Table 1). The highest level of inhibition (15 mm) was recorded for isolate from fresh milk against *Pseudomonas aeruginosa* (Plate A). The result obtained contradicts the earlier report of Gilliland and Speck (1977). These workers showed that *Lactobacilli* showed stronger antibacterial properties against Gram - positive than Gram - negative bacteria.

Recently, the antibacterial effect of *Lactobacillus* isolates have been demonstrated against *Salmonella* and *E.coli* by agar diffusion / spot methods (Oyarzabal and Conner 1995; Jim *et al* 1996). The ability of the isolate to antagonise the growth of these indicator bacteria as observed in the present report is a good index that these strains can produce metabolic products that can inhibit the growth of pathogen in the intestine. Juven *et al* (1992) reported that strains of *L. acidophilus*147 from chicken intestine produce lactic acid, hydrogen peroxide and a bacteriocin. All these substances have antibacterial properties. Figure 1, shows the plates (A & B) of the inhibitory effect of *L.acidophilus* strains on some of the indicator bacteria.

The four *L.acidophilus* strains were able to adhere to the IEC of albino rat. The highest level of adhesion of 23 bacteria/cell was recorded for isolate from albino rat while the least was observed for isolate from pig (5 bacteria/cell) (Table 2). Several workers have reported the host specificity of bacteria strain adhesion to epithelial cells of chicken crop, rats and pig

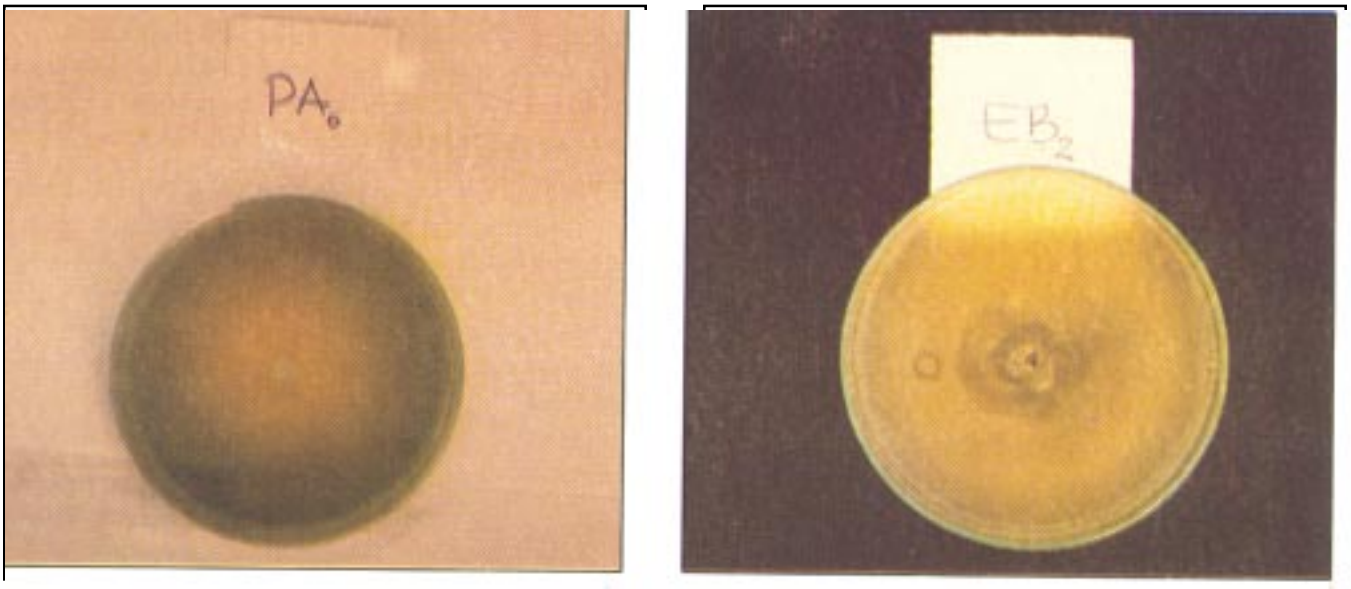

Fig 1. Inhibition of bacteria indicator by *L.acidophilus* strains. The indicator of plate 'A' is *Pseudomonas aeruginosa* NCIB 950 while plate 'B' is *Bacillus cereus* NCIB 6349.

sqamous *in vitro* (Fuller 1975; Suegara *et al* 1975; Barrow 1980). The adhesion ability varies between bacterial species and even different strains of the same species show variations (Fuller 1986). This may account for the high adhesion recorded for strain isolated from albino rat faeces.

A temperature of 37˚C was used to ascertain the adhesion capability of the different strains to the IEC of albino rat. Fuller (1975) had earlier reported that temperature in the range of 4˚C to 47˚C has no effect on the ability of *Lactobacilli* to adhere to the IEC of chicken, hence a temperature of 37˚C was adopted. A contact time of 1h (more than 30 mins) was adopted as recommended by Jin *et al* (1996). There was a significant difference ($P< 0.05$) in the adhesion of the different strains to albino rat IEC.

This study shows that wild strain of *L.acidophilus* from the faeces of human, pig and albino rat can produce antibacterial agent against major pathogenic bacteria. It also reveals that different strains of *L. acidophilus* have varying degree of adhesion to the IEC of albino rat with the isolate from albino rat having the highest adhesion.

***Key words*:** Probiotic, *Lactobacilli,* Antagonism, Adhesion.

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