HEALTH IMPORTANCE OF FAECAL STRAINS OF *Lactobacillus acidophilus* USED AS PROBIOTICS IN RATS

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Abstract

The health promoting potential of Lactobacillus acidophilus isolated from faeces of human neonate, pig and albino rat was assessed. A set of rats were orogastrically dosed with the Lactobacillus isolates alone (safety test), while the other set was dosed with Lactobacillus isolates and infected with E. coli NCIB 86 (Challenge test). The feeding period lasted for 20 days. The live weight and the feed efficiency of the rats were recorded before they were killed. Blood samples were collected and analysed for some serum biochemical markers that can reveal toxicological effect. The study showed that Lactobacillus acidophilus from the sources above has no toxicological effect on rats. Most of the serum biochemical markers increased significantly ($P < 0.05$) in the challenge test when compared to the safety test. Furthermore, the anticholesterolaemic effect observed in the safety test was impaired in challenge test. Feed efficiency was higher in rats dosed with Lactobacillus and challenged with E. coli.

Keywords: Faecal strain, *Lactobacillus acidophillus*, health importance, probiotics, rats.

1. Introdution

Probiotics have been defined as viable microbial food supplements, which beneficially influence the health of the host (Schrezemeir and De Vrese, 2001). Metchnikoff (1907) first observed the beneficial effects of living microorganisms on human and since then much interest had been on the beneficial effects of living microorganisms on human and animals (Fuller, 1989). Probiotic organisms have been found mainly among the members of the genera *Lactobacillus* and *Bifidobacterium*. These are normal residents of the complex ecosystem of the gastrointestinal tract (Mitsuoka, 1992).

Lactobacilli have been used as biotherapeutic agents for ages and they are still the most common ingredients among those intended for consumption by farm animals (Silva *et al.*, 1999). The choice of lactobacilli as probiotic agents is appropriate since the normal gastrointestinal microbiota of man and animals is rich in this organism (Tannock, 1977). Moreover, they are non-pathogenic microorganism (Sandine, 1979).

Several beneficial effects of probiotic *lactobacilli* have been documented. Some of these health promoting effects are: prevention of gastrointestinal infection (Tannock *et al.*, 1988), enhancement of immune response (Kimura *et al.*, 1997), antimutagenic and anticarcinogenic activity (Fuller and Gibson, 1997; Zabala *et al.*, 2001), anticholesterolaemic and liver improvement functions (Bertazzoni *et al.*, 2001).

Current perspective on biotechnical applications of probiotic products require further in-vitro and in-vivo investigation to evaluate the safety of using wild type organisms or those obtained by genetic engineering (Walker and Duffy, 1998). Probiotics normally form part of the diet. The relationship between diet and disease can be revealed by biomarkers since they provide a link between the consumption of specific foods and biological outcome (Branca *et al.*, 2001). The activities of these biomarkers can therefore be used to ascertain the health promoting effect of probiotics.

The objective of the research reported here was to establish the health promoting properties of different strains of *Lactobacillus acidophilus* isolated from faeces of human neonate, pig and albino rat.

2. Materials and Methods

Lactobacillus culture

Three strains of *Lactobacillus acidophilus* were isolated from the faeces of a human neonate, a pig and an albino rat. These isolates had earlier been characterised using colonial morphological, biochemical, and RAPD-PCR. Preliminary studies reveal that these isolates can inhibit pathogenic and food spoilage bacteria, and can also adhere to the ileal cells of rats *in - vitro*.

The *Lactobacillus* isolates were cultured in MRS broth (LAB M) and incubated at 37 ^{0}C for two days in a fermentor to obtain a large cell concentration. The method described by Fujiwara *et al.* (2001) was used. The cells were washed and resuspended in rehydrated skim milk (Marvel brand) (10% w/v), lyophilised and stored at –20 ^{0}C until use. The concentration of the viable cells in the final powder was determined by serial dilution and plating on MRS agar (Taylor, 1962).

Animals and Diet

Thirty-two albino rats (Wistar strain) aged 5 – 6 weeks old were obtained from the Physiology Department, University of Ibadan. The rats were housed at the Federal University of Tech-

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nology, Akure rat house and maintained at 27±1°C. The rats were fed on basal diet - grower's mash purchased from Bendel Feeds, Edo State, Nigeria, for 1 week ad libitum before the treatment. The composition of the basal diet is shown in Table 1.

Experimental Design

Lyophilised *Lactobacillus* isolates were reconstituted by dissolving 1g in 10ml of sterile water. The safety test involve a single dose of 0.3ml of these cultures containing approximately 10^{10} cfu/g administered to 4 rats in groups 1AS, 1BS, 1PS while group C that serves as control, was given sterile skim milk only (Bertazzoni *et al.* 2001). The challenge test involves dosing with *Lactobacillus* isolates and simultaneously infecting with 10^{5} cfu/ml of *E. coli* NCIB 86 in groups 1AC, 1BC and 1PC. The same procedure was followed the second day for the safety and challenge tests. A post - ingestion period of 18 days was observed after the administration of the cultures.

The initial weight, final weight, and feed consumed were recorded before the animals were sacrificed by cervical dislocation. The blood samples of the rats were collected into EDTA bottles for analyses of major serum biomarkers.

Biochemical Assay

Reflotron M06.02<06.00 (Boehringer Mannheim Company, Germany) was used for the analyses of some major serum biochemical markers that can reveal the effects of the administered culture on the rat. The biomarkers assayed for are: Total bilirubin, Aspartate-aminotransferase (AST), Alanine-aminotransferase (ALT), Alkaline phosphatase (ALP) and total cholesterol of the serum. Standardized amounts of the sample were automatically pipetted and applied on the test zone of the appropriate test strip. The strip was inserted into the test chamber and the result was displayed after some seconds on the computer monitor. Tests were carried out at 25°C.

Statistical Analysis

Data were analysed using the one-way ANOVA method, followed by Duncan test using SPSS 10.0 package. The level of significance was taken as $P< 0.05$. The results were reported as mean of 4 rats per set and their standard deviation calculated.

3. Results and Discussion

Lactobacillus acidophilus sourced from the faeces of human neonate, pig and albino rat were selected primarily because preliminary test shows that they have two major probiotic properties of inhibiting pathogenic bacteria and being able to adhere to the ileal epithelial cell (IEC) of the rat. These probiotic strains are expected to exert a beneficial effect on the host.

The relationship between diet and diseases or good health can be ascertained by biomarkers, and major biomarkers such as plasma enzymes viz: AST, ALT, ALP etc, changes in disease and can be related in many ways to cell pathology (Baron *et al.*, 1994). The serum AST was significantly higher ($P < 0.05$) in groups 1BC (297.73 iu/l) and 1PC (564.00 iu/l) when compared with the other groups (C, 1AS, 1BS, 1PS, and 1AC) that have AST level between 85.93 and 119.67 iu/l (Table 2). The serum AST activity was not significantly different ($P<0.05$) in groups' 1AS, 1BS and 1PS when compared with the control (C). Moreover, the ALT activities was significantly higher ($P < 0.05$) in groups 1AC, 1BC and 1PC (Table 2). The AST and ALT are enzymes that are located in the liver cells and leak out into the general circulation when liver cells are injured. Alanine aminotransferase (ALT) is regarded as a more specific indicator of liver inflammation, since the AST may be elevated in diseases of other organs such as the heart and muscle (Johnston, 1999). The implication of the result above is that in the safety test, there may not be any toxicological effect but in the challenge test, there is likely to be toxicological effect because of the elevated ALT.

Alkaline phosphatase (ALP) activities of the serum also reveal a significant difference ($P < 0.05$) in the level of this enzyme in groups that were dosed with *Lactobacillus* isolates and simultaneously infected with *E. coli* (Table 2). An increase in osteoblastic activity had been linked with a rise in ALP level (Baron *et al.*, 1994). The ALP activity can also serve as an indicator of liver damaqge when there is lack of bile flow (cholestasis). In essence, feeding with *Lactobacillus* alone in the safety test has the potential of reducing the incidence of cholestasis as observed in groups 1AS, 1BS, and 1PS.

The serum cholesterol level was significantly higher ($P < 0.05$) in groups 1AC, 1BC and 1PC than the control (C) (Fig. 1). Fuller (1989) had suggested that bacterial metabolites in fermented milk inhibit cholesterol synthesis. Lactobacilli in particular had been found to have direct effect on cholesterol level by assimilation and removal from the growth medium. This has been demonstrated in pigs (Gilliland *et al.*, 1985) and in rats (Bertazzoni *et al.*, 2001). Studies have indicated that the serum cholesterol level is one risk factor in the incidence of coronary artery disease and individuals with elevated serum cholesterol values develop coronary heart disease (CHD) with greater frequency (Kannel, 1978). In essence when these *Lactobacillus* isolates were administered alone they possess anticholesterolaemic effect but this effect was impaired in the challenge test. The lower level of serum AST, ALT, and cholesterol observed in groups' 1AS, 1BS, and 1PS when compared to the other treatments shows that a high dose of the probiotic (10^{10} cfu/g), when applied twice, can persist for up to three weeks or more after administration. Pascual *et al.* (1999) had earlier suggested that more than one dose would be necessary to ensure the presence of *Lactobacillus salivarius* in the gut of birds for 21 – 28 days.

Growth performance measurements reveal that the total weight gain (TWG) of the rats in groups' 1AC, 1BC and 1PC was higher than the control (C) and the groups' fed with *Lactobacillus* alone (Table 3). The results shows that groups 1AC, 1BC and 1PC had the highest ALT activities and cholesterol level. The high level of serum ALT and cholesterol in the groups' above may be responsible for the

Table 1: Composition of Basal Diet Used In Feeding Rats.

Ingredients	Level in Diet
Crude protein	14.5%
Crude fat	4.8%
Crude fibre	7.2%
Crude ash	8.0%
Calcium	0.8%
Phosphorus	0.62%
Lysine	0.60%
Methionine	0.29%
Methionine + Cystine	0.52%
Vitamin A	8,000 i.u
Vitamin D	2,400 i.u
Vitamin E	15mg
Vitamin B_2	40mg
Vitamin C	50mg
Manganese	30mg
Zinc	30mg
Sodium	0.15%

Metabolisable energy: 2,300 kcal./kg
Source: Bendel Feeds, Edo State, Nigeria.

Table 2: Level of Biochemical Markers in the Serum of Rats After *In-vivo* Feeding.

Isolates	AST (iu/l)	ALT (iu/l)	ALP (iu/l)
C	93.47±66.66	31.43±9.47	32.90±0.06
1AS	70.97±13.77	33.03±3.89	32.93±0.06
1BS	119.67±16.44	33.87±8.29	32.90±0.06
1PS	107.03±36.02	15.87±5.18	32.93±0.06
1AC	85.93±4.00	78.80*±33.29	973.53*±92.20
1BC	297.33*±36.05	76.47*±8.60	961.67*±118.35
1PC	564.00*±29.30	85.33*±12.42	1005.33*±221.61

Results are expressed as the mean ± Std. Deviation for 4 animals per group.
*Values along the column are significantly higher (P <0.05) than the control (C).
C: Rats fed basal feed only (C)
1AS: Rats fed *Lactobacillus acidophilus* sourced from rat faeces
1BS: Rats fed *Lactobacillus acidophilus* strain sourced from human neonate faeces
1PS: Rats fed *Lactobacillus acidophilus* strain sourced from pig faeces
1AC: Rats fed *Lactobacillus acidophilus* strain sourced from rat faces and challenged with *E. coli*
1BC: Rats fed *Lactobacillus acidophilus* strain sourced from human neonate and challenged with *E. coli.*
1PC: Rats fed *Lactobacillus acidophilus* strain sourced from pig faeces and challenged with *E. coli.*
AST: Aspartate aminotransferase
ALT: Alanine aminotransferase
ALP: Alkaline phosphate

Table 3: Performance of Rats After *In-vivo* Feeding.

Strain Designation	Feed consumed	Total Weight Gain	Feed Efficiency
C	400 ± 1.21	36.50±5.29	0.091±0.06
1AS	396.63 ± 2.04	36.50±2.78	0.092±0.07
BS	397.00 ± 0.57	24.83±1.25	0.062±0.06
1PS	396.13 ± 0.55	35.50±13.59	0.089±0.06
1AC	395.4 ± 2.02	57.17*±9.27	0.144*±0.05
1BC	396.77 ± 1.25	53.50*±12.12	0.134*±0.05
1PC	396.33 ± 0.88	53.50*±7.00	0.134*±0.09

Results are expressed as the mean ±Std. Deviation for 4 animals per group.
*Values along the column are higher and significantly different (P < 0.05) from the control (C).

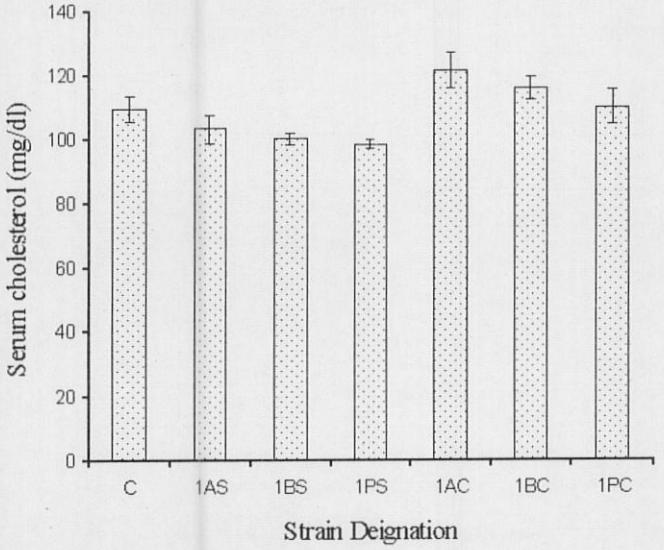

Fig 1: Serum Cholesterol of Rats after in - vivo Feeding Trials.

higher weight. The correlation between serum cholesterol, ALT, and weight had been reported (Johnston, 1999). Low cholesterol had been found to result to reduced weight and low ALT activity. Assessment of the feed efficiency also reveals that group 1AC had a better performance than the other groups. In terms feed utilisation, groups 1AC, 1BC, and 1PC would consume 56.62%, 46.57% and 46.55% respectively less feed to achieve the same liveweight as control (C). The feed utilisation in groups 1AS and 1PS were not significantly different from the control but it was significantly different in 1BS (Table 3).

These results showed that faecal strains of *Lactobacillus acidophilus* possess health-promoting activities such as anticholesterolaemic effect and liver function improvement when administered alone. The feed efficiency was however lower in rats dosed with *Lactobacillus species* alone but higher in rats dosed with *Lactobacillus species* and simultaneously challenged with *E. coli.* This may be due to the higher level of cholesterol and ALP in the serum of these rats in challenge test.

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