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Assessment of the Safety of Wild Strains of *Lactobacillus* as Probiotics Orogastrically Administered to Rats

V. O. Oyetayo

Department of Microbiology, Federal University of Technology, PMB 704, Akure, Nigeria

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Abstract. The safety of four wild strains of *Lactobacillus,* isolated from fresh cow milk and faeces of albino rat (*Rattus norvegicus*) was studied. Some biochemical parameters of the serum in the orogastrically-dosed rats were used as the index. A reduction in the levels of serum cholesterol and of serum aminotranferases in the rats orogastrically-dosed with *Lactobacillus* isolates, as compared with the control group was noted. There was no significant difference ($P > 0.05$) in the alkaline phosphatase levels of the control and the orogastrically-dosed rat groups. Serum globulin and bilirubin levels showed a significant difference ($P < 0.05$) among the control and the *Lactobacillus*-dosed groups. The control group recorded the highest weight gain among all the groups studied, but it was not significantly different ($P > 0.05$) from other treatments except in the rats dosed with the *Lactabacillus casei* strain isolated from cow milk. The rats dosed with *Lactobacillus* displayed beneficial effects as probiotics in terms of reduced serum cholesterol and liver function improvement in terms of reduction in the serum aminotransferase levels.

Keywords: *Lactobacillus* isolates, serum cholesterol, albino rats, cow milk, probiotics, *Lactobacillus* safety

Introduction

Probiotics are the food supplements containing viable microbes, which have the potential to beneficially influence the health of the host (Schrezenmeir and De Vrese, 2001). Metchnikoff (1907) observed, at the beginning of the last century that the consumption of fermented foods was helpful in controlling autointoxication caused by the putrefactive microbial species that produce toxic compounds in the intestine. Attempts have been made to replace this putrefactive microbiota with the saccharolytic species. *Lactobacillius, Streptococcus*, and *Bifidobacterium* species have been found to play a useful role in this respect (Fuller, 1989). Species belonging to *Lactobacillus* have been reported to play an important role in the maintenance of the intestinal ecosystem (Sandine, 1979). These organisms have been shown to possess antagonistic effect towards the growth of pathogenic bacteria, such as *Listeria monocytogenes*, *Escherichia coli,* and *Salmonella* spp. (Drago *et al.,* 1997; Chateau *et al.,* 1993; Ashenafi, 1991). The inhibitory properties of the *Lactobacilli* have been linked with metabolic products, such as organic acids (mainly lactic acid), hydrogen peroxide, and bacteriocins (Juven *et al.,* 1992).

An essential determinant in the choice of a probiotic microorganism is its ability to reach, survive, and persist in the environment in which it is intended to act (Marteau *et al.,* 1992). Some workers have demonstrated the survival and temporary colonisation of the human gastrointestinal tract by some lactic acid bacteria (Jacobsen *et al.,* 1999; Alander *et al.,* 1997). Walker and Duffy (1998) pointed out that the current perspective on biotechnical applications of probiotic products requires further *in vitro* and *in vivo* investigations to evaluate the safety of using wild-type organisms, or those obtained by genetic engineering. The safety and health promoting effect of *Lactobacillus casei* isolated from different sources, mainly the European cheeses, have been reported by using biomarkers of serum and faeces (Bertazzoni *et al.,* 2001). The relationship between diet and disease/health can be revealed by these biomarkers, since they provide a link between the consumption of specific foods and the biological outcome (Branca *et al.,* 2001). Major biomarkers, such as plasma enzymes, and their changes during the disease are related in many ways to cell pathology (Baron *et al.,* 1994). The aim of the present study was, therefore, to assess the safety of wild strains of *Lactobacillus,* isolated from fresh cow milk and faeces of albino rats, when orogastrically-dosed to rats using biochemical markers in the serum as the index.

Materials and Methods

***Lactobacillus* cultures.** Four *Lactobacillus* strains, isolated from fresh cow milk and faeces of albino rats, were used in the present study (Table 1). These isolates were characterised using the colony, morphological and biochemical characteristies. The isolates were observed to show antagonistic effects against some pathogens and had the ability to adhere

E-mail: ovofuta@yahoo.com

Table 1. The source and strain designation of *Lactobacillus* species used for orogastric administration in rats

Source of strains isolated	Strain designation	*Lactobacillus* species identified	Cell count (cfu/g)*
Fresh cow milk	1 M	*L. acidophilus*	5×10^{10}
Fresh cow milk	2 M	*L. casei*	4×10^{10}
Rat faeces	1 A	*L. acidophilus*	9×10^{10}
Rat faeces	2 A	*L .brevis*	9×10^{10}

cfu/g = colony forming units per gram; *mean of three replicates

to the ileac epithelial cells of the albino rats (Oyetayo *et al.,* 2004). The *Lactobacillus* isolates were cultured in MRS broth (LAB M) and incubated at 37 °C in an anaerobic environment for 48 h to obtain high cell concentration. The method described by Fujiwara *et al.* (2001) was used to harvest the cells by centrifuging at 10,000 xg for 15 min at 0 °C. The cells were then washed twice with sterile water. The washed cells were resuspended in dehydrated skim milk (10% w/v, Marvel brand), lyophilised in a lyophiliser, and stored at -20 °C until use. The concentration of viable cells in the final powder was determined by serial dilutions and plating on MRS agar medium in triplicate (Table 1).

Animals and diet. Twenty Wistar rats *(Rattus norvegicus)*, 5-6 weeks old, were obtained from Physiology Department, University of Ibadan, Nigeria. They were housed at the rathouse of the Federal University of Technology, Akure, Nigeria and maintained at 27±1°C. The rats were fed on basal diet, a product of Bendel Feed, Edo State, Nigeria, for one week *ad libitum* before the treatment. The composition of the basal diet is given in Table 2.

Orogastric administration of the *Lactobacillus* strains. The rats were divided into five groups of four rats each. The *Lactobacillus* isolates were reconstituted by suspending 1 g of the lyophilised culture containing approximately 10^{10} cfu/g in 10 ml of sterile water. A single dose of 0.3 ml of the culture suspensions of the four isolated strains (1M, 2M, 1A, 2A) was separately administered to 16 rats (4 rats for each *Lactobacillus* strain) by orogastric-dosing, while the remaining 4 rats that served as the control were administered sterile skim milk (Bertazzoni *et al.,* 2001). This treatment was repeated again on the second day. Post-ingestion period of 18 days was allowed to elapse after the administration of the *Lactobacillus* strains. The initial weight and final weight gain were recorded. At the end of the feeding test, the rats were killed by cervical dislocation and their blood aseptically collected into ethylenediaminetetraacetic acid (EDTA) bottles,

Table 2. Composition of basal diet used to feed the test albino 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 %
Sodium	0.15 %
Manganese	30 mg
Zinc	30 mg
Lysine	0.6 %
Methionine	0.29 %
Vitamin A	8,000 iu
Vitamin D-3	2,400 iu
Vitamin E	15 mg
Vitamin B-2	40 mg
Vitamin C	50 mg
Metabolisable energy	2,300 kcal/kg

*source: Bendel Feed, Edo State, Nigeria

specified for conducting the biochemical analyses of the serum.

Biochemical assays. Reflotron M06. 02 < 06.00 kit (Boehringer Mannheim Company) was used for biochemical analyses of serum to study the effects of the administered *Lactobacillus* strains. The biomarkers assayed were: total bilirubin (Bil), total cholesterol (Chol), aspartate aminotransferase (Ast), alanine aminotransferase (Alt), alkaline phosphatase (Alp), and globulin (Glob) levels of the serum. Standardized amount of the sample was applied on to the test zone of the appropriate test strip. The strip was inserted into the test chamber and the flap closed. The analytical results were monitored after some seconds on the computer monitor. All the tests were carried out at 25 °C.

Statistical analysis. Data were analysed by one-way ANOVA followed by Dunnet tests using SPSS 7.5 package. The level of significance was taken as $P < 0.05$. The results were reported as mean values.

Results and Discussion

The strains used for this feeding trial were selected on the basis of their inhibitory potential as shown on some pathogenic bacteria and their ability to adhere to the intestinal epithelial cells of the experimental albino rats (Oyetayo *et al.,* 2004). These strains of *Lactobacillus* were expected to exert a beneficial effect on the maintenance of the intestinal micro-flora of the host animals (Chang *et al.,* 2001).

Table 3. Levels of some biochemical markers in the serum of albino rats, orogastrically-dosed with various strains of *Lactobacillus* species isolated from cow milk and rat faeces

Rat test group	Biochemical markers in the rat serum*				
	Alt (iu/l)	Ast (iu/l)	Alp (iu/l)	Bil (mg/l)	Glob/mg/l)
lA (*L. acidophilus*)	25.87^{ab}	94.98^{a}	32.92^{a}	0.47^{c}	24.75^{a}
2A *(L. brevis)*	13.06^{a}	168.20^{a}	32.67^{a}	0.85^{ab}	23.50^{a}
1M (*L. acidophilus*)	18.08^{bc}	132.00^{a}	32.67^{a}	0.83^{ab}	21.00^{ab}
2M (*L. casei*)	14.93^{cd}	135.00^{a}	32.92^{a}	0.83^{ab}	21.00^{ab}
Control	29.65^{ab}	251.03^{a}	32.90^{a}	0.63^{bc}	23.50^{a}

Alt: alanine aminotransferase; Ast: aspartate aminotrasferase, alkaline phosphatase; Bil: bilirubin; Glob: globulin; 1A and 2A: isolated from rat faeces; 1M and 2M: isolated from cow milk; i u: international units; *values are mean for four rats/treatment and the same superscripts along the column are not significantly different at $P < 0.05$

The final weight gain was slightly higher in the rats of the control group than the rats in all the four test groups that were orogastrically administered the isolated *Lactobacillus* strains (Fig.1). There was no significant difference ($P > 0.05$) in the weight gain of rats in the control group and the test groups 1A, 2A and 1M, however, differences were significant ($P < 0.05$) in the group 2M. The control group consumed more feed (401 g) as compared with the other test groups which consumed 394-398 g of the feed. This difference in the feed consumption was not reflected in the weight gain difference between the control and the other test groups, except in the 2M group. The level of alanine aminotransferase (Alt) has been reported to decline with weight loss (Johnston, 1999). The present report shows that there was a correlation between the weight gain and the Alt level in the serum of the rats. Rats in the control group had higher weight and higher serum Alt than the other test groups (Table 3). The serum cholesterol was observed to be higher in the control group (107.75 mg/100 ml) than the other groups (Fig. 2). It has been suggested that bacterial metabolites in the fermented milk inhibited cholesterol synthesis (Fuller, 1989). Bertazzoni *et al.* (2001) recently reported a slight reduction in the serum cholesterol of rats treated with *Lactobacillus casei,* sourced from the European cheese. The *Lactobacilli* used in this study also showed the ability to reduce serum cholesterol, and in effect it may be concluded to have the potential of reducing the incidence of coronary heart disease (CHD). Studies have indicated that serum cholesterol level is a risk factor in the incidence of CHD and that persons with elevated serum cholesterol values develop CHD with greater frequency (Branca *et al.,*2001; Kannel, 1978). The levels of aminotransferases (both Alt and Ast) were higher in the control group as compared with test groups. The increased levels of Ast and Alt reflected active hepatocellular damage (Baron *et al.,* 1994). The level of Alt was generally lower than

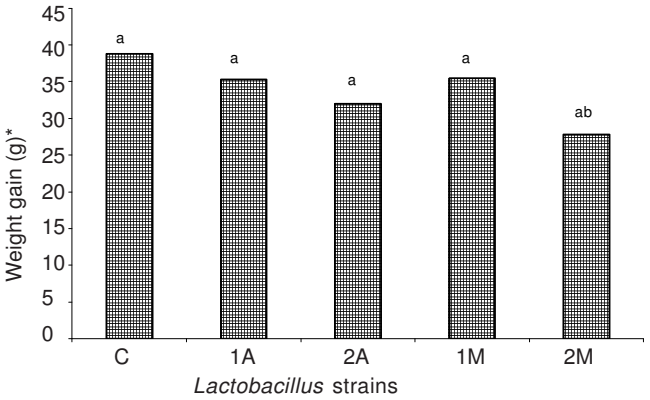

Fig. 1. Final weight gain of rats orogastrically-dosed with *Lactobacillus* isolates from various sources (C: control; 1A and 2A, respectively, *L. acidophilus* and *L. brevis* isolated from rat faeces; 1M and 2M, respectively, *L. acidophilus* and *L. casei* isolated from cow milk;
*bars with the same letter are not significantly different ($P > 0.05$).

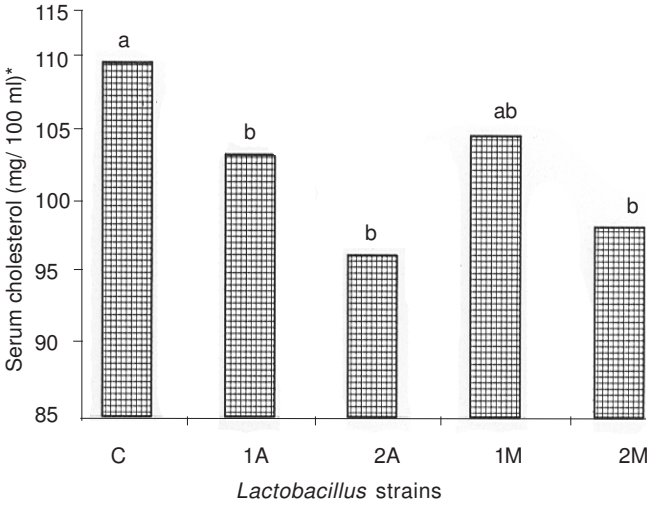

Fig. 2. Level of serum cholesterol in rats orogastrically dosed with *Lactobacillus* isolates; *bars with the same letter are not significantly different ($P < 0.05$).

Ast. Alanine aminotrasferase (Alt) is more specific for monitoring the liver damage, since it is principally found in the liver (Johnston, 1999; Baron *et al.,* 1994; Cheesborough, 1991), while other organs such as kidney, cardiac muscles, skeletal muscles, etc., can produce Ast. The lower Alt and Ast levels founds in the serum of the rats dosed with the *Lactobacillus* species is an indication of improved liver function. These observations indicated that *Lactobacillus* species can still persist in the gostrointestinal tract of the rats for up to three weeks. There was no significant difference ($P < 0.05$) in the level of Alp in all the rat trial groups. An increase in osteoblastic activity has been linked with a rise in the Alp level (Baron *et al.,* 1994). A rise in Alp level can also occur when there is a lack of bile flow (cholestasis). In the present study, there was no significant difference ($P > 0.05$) in the level of Alp in the serum of the experimental rats, when compared with the control group (Table 3).

Immunoglobulins are most often investigated in children sufferring from recurrent infections, or a combination of infections with allergy (Branca *et al.,* 2001). The globulin level was slightly higher in the serum of rats dosed with *Lactobacillus acidophilus* isolated from albino rat faeces. The ability of *Lactobacilli* to cause a non-specific immune response, when ingested, has been reported (Kimura *et al.,* 1997). This immunostimulatory potential was, however, not displayed by the other *Lactobacillus* isolates.

The bilirubin level in serum is also a useful indicator of pathological effects. Over-production of bilirubin has been linked with an excess breakdown of red blood cells (Cheesborough, 1991). This may be due to many factors, one of which is the toxins produced by bacteria. The lowest level of bilirubin was observed in rats treated with strain 1A (*L. acidophilus*) isolated from albino rat faeces. The lower bilirubin level in rats, dosed with the strain 1A, may be an indication of the specificity of the isolate to the host; hence it is amiable. Chang *et al.* (2001) reported recently that *Lactobacillus reuteri* BSA 131 from pigs had a better potential as probiotic agent for piglets, especially after weaning.

The results obtained during the present study reveal that oral administration of wild strains of *Lactobacillus* isolated from fresh cow milk and albino rats is safe. These isolates have also been noted to show beneficial effects due to their anticholesterolaemic activity and reduction in the aminotransferase levels, which is an indication of improved liver function. These observations indicate the probiotic potential of these strains of *Lactobacillus* species.

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