Immunostimulatory and Protective Properties of *Lactobacillus brevis* Used as a Biocontrol Agent *in Vivo*

Agarry, O. O.

Department of Biological Sciences, Microbiology Unit, University of Abuja , P. M. B. 117,Abuja, Nigeria
E-mail: oluagarry@yahoo.com

Abstract: The immunostimulatory and protective properties of Lactobacillus brevis isolated from cassava starch were studied in vitro and in vivo. Antagonism was measured by the zone of inhibition between the bacterium streak/ring and fungus plug. Subsequent increases in inhibition were observed and complemented by a small but progressive decrease in the distance between the bacterium and the fungus. L. brevis significantly (>74%) inhibited the growth rate of Fusarium moniliforme after 168 h. Biochemical indices of albino rat plasma showed that the bacterium had liver improvement functions. Plasma aspartate aminotransferase (AST) activity of the rats dosed with L. brevis alone was lower (8.33 IU/L) than the control. A mild elevation of AST and alanine aminotransferase (ALT) activities was observed in rats administered with L. brevis and F. moniliforme implying that the bacterium possesses antimycotic properties capable of reducing the severity of pathogen attack on the host. However, there was a significant ($P<0.05$) decrease in the plasma globulin and protein levels. There was a reduction in the count of F. moniliforme in rats dosed with both organisms during feeding trials. The weight gain by rats in the treatment group compared favourably with the control. Further pathological investigation confirmed a pale and friable liver while the small intestine was inflamed. The administration of L. brevis had and immunostimulatory effect. Lactobacillus brevis has not only potent in vitro antifungal activity against F. moniliforme but also in vivo control efficacy against Fusarium infection. Further evaluation of its effectiveness for disease control and applications should be done

[Agarry, O. O. Immunostimulatory and Protective Properties of *Lactobacillus brevis* Used as a Biocontrol Agent *in Vivo*. Journal of American Science 2010;6(11):1038-1045]. (ISSN: 1545-1003). (http://www.americanscience.org)

Keywords: Immunostimulatory properties, Fusarium moniliforme, Lactobacillus brevis

INTRODUCTION

Lactobacillus brevis is a species of lactic acid bacteria. Ingestion has been shown to improve human immune function, and it has been patented several times (Yoshindo, 2005). While interferon is attracting international attention as a specific medication for the treatment of cancers and viral diseases, *Lactobacillus brevis* has been observed to increase the production of interferon in the body (Akihiko, 1994; 1995). In addition to strengthening the specific immunity, lactic acid bacteria also seem to reinforce the non-specific mechanisms of defense such as phagocytosis and cytokine production. Secretion by these organisms of compounds having anti-inflammatory or antimicrobial effects has also been suggested (Heyman, 2000). *Fusarium* is one of the emerging causes of opportunistic mycoses (Anaissie *et al*., 1988; Guarro and Gene, 1995). Infections due to *Fusarium* spp. are collectively referred to as fusariosis. Outbreaks of nosocomial fusariosis have also been reported. Existence of *Fusarium* in hospital water distribution systems may result in disseminated fusariosis in immunosuppressed patients. *Fusarium* is one of the most drug-resistant fungi. *Fusarium* infections following solid organ transplantation tend to remain local and have a better outcome compared to those that develop in patients with hematological malignancies and bone marrow transplantation patients (Deshpande and Koppikar, 1999; Tanure *et al*., 2000 and Schell, 2000).

The colonic microflora microflora is important to health. The growth and metabolism of the many individual bacterial species inhabiting the large bowel depend primarily on the substrates available to them, most of which come from the diet. The microflora normally presents a barrier to invading organisms; but pathogens often become established when the integrity of the microbiota is impaired through stress, illness, antibiotic treatment, changes in diet, or physiological alterations in the gut. Innovative approaches have been tried as alternative to antibiotics in treating gastrointestinal diseases and these include using live biotherapeutic agents such as yeast (*Saccharomyces* spp.) and bacterial isolates (*Lactobacillus* spp.) or faecal enemals (Fuller 1992).

Lactobacilli have been widely used in treating diarrhoeal diseases such as pseudomembranous colitis, but the results have been mixed. Feeding freeze-dried powders of *L. acidophilus* NCDO 1748 had no effects on patients with pseudomembranous colitis (Aronsson *et al*. 1987). The presence of this group of bacteria in the gut is considered to have several potential benefits such as protection from pathogens (Casas and Dobrogosz 2000), anticholesterolaemic effect (Bertazzoni *et al*. 2001) and immustimulation (Aattouri *et al*. 2001). However, not all lactobacilli are effective in combating enteric pathogens.

Fusarium is listed as one capable of causing mycetomas (Schell, 2000) and it has repeatedly been isolated from human keratitis (Deshpande and Koppikar, 1999) and corneal ulcers. Experimental animals often experience hepatotoxicity, nephrotoxicity or both; rats have also been shown to experience necrosis of stomach mucosa and myocardium due to the toxins, fumonisins. Liver cancers are induced. Fumonisins are also among the chief suspects for the agent(s) of elevated levels of esophygael cancer in certain parts of the world (Pitt, 2000).

In the current situation where the discovery of new antimicrobial agents are becoming increasingly difficult, the present study suggests that investigation of lactic aid bacteria may offer some potential applicability to chemotherapy.

MATERIALS AND METHODS

Microbial culture

Lactobacillus brevis and *Fusarium moniliforme* were isolated from cassava starch on de Mann Rogosa and Sharpe (MRS) agar and Malt Extract Agar (MEA). All growth media were supplied by Oxoid (Melbourne, Australia). The isolates were characterized using colonial, morphological and biochemical methods.

In vitro antifungal activity

Modified methods of Fokkema (1973) and Adetuyi and Cartwright (1985) were used for the detection of antagonistic activity of bacterial isolate towards the growth of the fungal culture as adapted by Agarry and Osho (2005).

In vivo feeding

Sixteen albino rats (Wistar strain) Aged 6-8 weeks were obtained from the Department of Biochemistry, University of Ilorin, Nigeria. The rats were fed on basal diet broiler starter (Amo-Byng Feeds and Concentrates, Oyo State, Nigeria). They were randomly assigned to 4 treatment groups designated as BUU (Basal diet only, uninfected with fungus and untreated with bacterium (control)), BUT (Basal diet, uninfected with fungus but treated with bacterium), BIU (Basal diet, infected with fungus and untreated with bacterium), and BIT (Basal diet, infected with fungus and simultaneously treated with bacterium , and each was made up of 4 rats per group. Lyophilised *Lactobacillus* cells were reconstituted by dissolving 1 g in 10 ml of sterile water.

Adult Wistar albino rats were held under specific pathogen free conditions. Group BUU animals were kept on the basal diet alone (control). Animals in Groups BUT and BIU were fed on the basal diet and were orogastrically dosed with *L. brevis* (0.3 ml) and *F. moniliforme (0.3ml)* respectively. Group BIT were fed with basal diet, orogastrically challenged with 10^8 cfu/spores of *F. moniliforme* and then treated with the administration of 0.3 ml of *L. brevis* (10^5 cfu/g). . The above treatments were repeated for a second day and a post-ingestion period of 14 days was observed after the administration of the cultures. Rats were then killed by cervical dislocation and blood samples collected into EDTA bottles for analysis of some plasma biochemical markers. The liver kidney, spleen, stomach and small intestine were removed for examination.

Biochemical assay

The biomarkers assayed for: aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), albumin, globulin and total proteins were conducted according to the conventional methods reported by Mokady *et al*. (1989). The haematological parameters namely packed cell volume (PCV), haemoglobin (Hb) count, white blood cell count (WBC) and differential counts were conducted using the methods of Aning *et al*. (1998).

Monitoring the progress of infection and faecal levels of *A. fumigatus*

The body weights of animals were recorded daily up to 14 days post-pathogen challenge. The data gathered were used to calculate the following parameters: (i) weight gain = final weight – initial weight (ii) percentage weight gain = weight gain/final gain x 100.

For enumeration of viable faecal *Aspergillus fumigatus*, freshly voided faecal pellets were collected and pooled from each rat (0.3-0.4 g per rat) at 1, 2, 7 and 14 days post-dosing (Chang *et al*. 2001). Faeces were weighed and homogenized. Faecal homogenates were serially diluted in sterile water, and a0.1 ml aliquot was added in duplicate onto MEA plates. Plates were incubated in aerobic condition for 4 days at 25°C. Colonies were

characterized on the basis of morphology and pigmentation. The population levels were converted to log values before plotting out in graphs.

Histopathological tests

At autopsy the internal organs were inspected for morphological lesions. Samples of the liver, kidney, stomach, spleen and small intestine from each animal were fixed in 10% formalin, dehydrated in different percentages of alcohol, cleared in xylene for 2 h and impregnated in liquid for 2 h for embedding. The embedded organs were sectioned to 2µm using a microtome and stained with haematoxylin eosin (Silva *et al*. 1999).

Statistical analysis

Results are expressed as means ± standard error of the mean. For statistical comparison, the data gathered were processed by one-way analysis of variance (ANOVA), SPSS 10.0. Means were compared by Duncan Multiple Range Test Statistical analysis was conducted with the Statistical Analysis System for personal computers (SAS Institute, Cary, NC, USA) software with the level of significance set at $p < 0.05$. Ethical declaration The study protocol was conducted in accordance with internationally accepted principles (European Community guidelines EEC Directive of 1986, 86/609/eec; US guidelines, NIH publication ~~H~~85-23, revised in 1985) for laboratory animal use and care.

RESULTS AND DISCUSSION

Antifungal activities

Lactobacillus brevis inhibited the growth of food spoilage and phytopathogenic fungus, *Fusarium moniliforme* (**Plate l**).

Physical contact between the bacterium and the fungus mycelium was never observed during the incubation period. An uncolonized zone between the bacterium and fungus was maintained throughout suggesting that diffusible metabolites of bacterial origin were responsible for inhibition of mycelium growth (**Plate 1**). The bacterium was not directly lethal to the fungus under the conditions of the bioassay but strongly inhibitory to the mycelial growth and spore formation. The use of a separate control apart from the Fokkema "control" side of the culture in the Fokkema-type bioassay was made to judge its true presentation of normal unchallenged mycelial growth. When compared to the 'true' control plate which has no bacterial inoculum on it, there appeared to be no significant difference (Fig. 1)in the two controls. Measurements of growth rate inhibition showed variations between the two techniques, and overall levels of activity were essentially similar (**Fig. 1**) but the streak bioassay of Fokkema (1973) method gave an erratic pattern of inhibition over the bioassay period than did the concentric ring method of Adetuyi and Cartwright (1985). The points at which inhibition was first detected and at which complete cessation of fungal growth (streak method: 96 h; ring method: 72 h) occurred in the two methods were also different with . The ring bioassay detected inhibition earlier(48h) than the streak bioassay(72h) (**Fig 1**). It also facilitated measurements of the distance between the bacterium and the fungus, and allowed accurate determination of fungal colony radius, since inhibitory effects were exerted equally around the colony. *L. brevis* inhibited fungal growth up to >74% in the ring method and >92% in the streak method (**Fig 1**). Oyetayo (2006) reported the inhibition of growth of pathogenic and food spoilage bacteria by *L. plantarum*.

Hepatoprotective effect

Plasma biomarkers in experimental animals after orogastric dosing with *L. brevis* reveal a significant reduction in aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels as compared with those placed on basal diet and dosed with *F. moniliforme* (**Table 1**). Experimental animals often experience hepatoxicity, nephrotoxicity or both due to the toxins, fumonisins produced by *F. moniliforme*. Fumonisms are also among the chief suspects for the agent(s) of elevated levels of esophageal cancer in certain parts of the world (Pitt, 2000). The rise in AST in rats fed basal diet and *F. moniliforme* could be attributed to possible secretion of mycotoxins. Oboh *et al*. (2000) reported aflatoxin B1 to be implied in liver damage.

AST values were reduced in rats dosed with the bacterium more than control rats (**Table 1**). Plasma AST and ALT are important enzymes used in monitoring liver damage (Johnston 1999). An increase in the level of these enzymes in the serum/plasma is an indication of hepatocellular damage (American Liver Foundation 1995).

AST and ALT are enzymes located in the liver cells and leak out and make their way into the general circulation when liver cells are injured (David and Johnston 1999). Mild or moderate elevations of AST or ALT are non-specific and may be caused by a wide range of liver diseases (American Liver Foundation 1995, 1997; David and Johnston 1999)

There was significant change(Table 1) ($P<0.05$) in plasma albumin, globulin and protein of the albino rats in all groups when compared with that of the control diet. Albumin measures the main protein made by the liver and tells how well the liver is making this protein. Low albumin as reported for Group BIU animals (11.60g/dL) may be caused by acute or chronic inflammation or liver disease (David

and Johnson, 1999; Younossi and Mehta, 1998). Slight elevation of albumin in Group BUT animals indicated an increase in the protein production made by the liver. The bacterium has the ability to stimulate the immune system of the rats. Albumin is produced mainly in the liver and therefore is a test of liver function. Low albumin levels and no other liver function test abnormalities are likely to result from a nonhepatic cause (David and Johnson 1999). Total protein measures albumin and other proteins in blood, including antibodies made to help fight off infections (Liver Function Tests 2005; Younossi and Mehta 1998).

Immunostimulatory effect

The WBC count increased in groups BIT and BUT (**Table 2**). This might result from the production of more white blood cells to engulf the antigen. T lymphocyte and other key cells of the immune system are known to activate production of antibody polymorphonuclear granulocyte to destroy an invading pathogen (Prescott *et al*., 1999). Differential leucocyte counts in Wistar rats dosed with *L. brevis* reveal a significant increase in neutrophil and ensinophil counts and mild decrease in lymphocyte counts as compared with the control (**Table 2**). The primary role of lymphocytes is in humoral antibody formation and cellular immunity (Baker and Silver 1985). An absolute increase in lymphocytes had been found in most bacterial infection such as Staphylococcal infection (Monica 2000). The administration of *L. brevis* to Group BUT animals had an immuno-stimulatory effect. Immunoglobulins are often sought in children with recurrent infections or a combination of infections with injury (Baron *et al*. 1994, pg 158). The PCV and Hb compared favourably with the standard (Mitruka and Rawnsley 1977; Baker *et al*. 1979; Weihe e 1987). Aning *et al*. (1998) and Oboh and Akindahunsi (2004) reported similar findings on the haematological parameters of albino rats fed sorghum and brewer's grains and albino rats fed *Saccharomyces cerevisiae*-fermented cassava flour diet. The PCV and Hb of the control diet and other groups were significantly different (P>0.05) suggesting that the treatment is not heamolytic. This result also agrees with Aletor (1993) to the extent that cassava products do not have negative haemotological effects. Agarry (2006) reported an improvement in blood composition of treated Wistar rats with an antagonistic pair of microbial isolates of cassava products origin. The improvement in blood composition that followed feeding of the animals with ()the bacterium indicated an immunological security for the group of animals given such treatment.(Table 2).

Other benefits

Animals singly dosed had fluctuating weight gain/loss over the 2-week period. The weight gain in the control group compared favourably with that in group BUT(Fig 2). This implies that the treatment enhanced the growth of the animals. A reduction of the faecal level of the fungus and increase in the level of the beneficial bacterium (*L. brevis*) was also observed (Fig 3). Animals of group BUT contained a low faecal number of pathogens. The faecal levels of the bacterium increased in animals of groups BUT and BIT. Although animals were doused with isolates for only 2 days after pathogen challenge, they were protected beyond that time(Fig 3). These observations are in line with observations made by Henriksson and Conway (2001), who demonstrated that a range of new bifidobacteria may provide protection against infection by *Salmonella typhimurium* in mice resulting in both an initial reduction of *S. typhimurium* levels in feaces and a reduced weight loss of animals challenged with the pathogen.

However, the pathological studies revealed possible damage to the internal organs of the animals. The small intestine, stomach and liver of rats showed significant lesions. Distention of issue parenchyma, cellular infiltration and partial erosion of the mucus membrane apparent while no significant lesions in the spleen and kidney was observed.

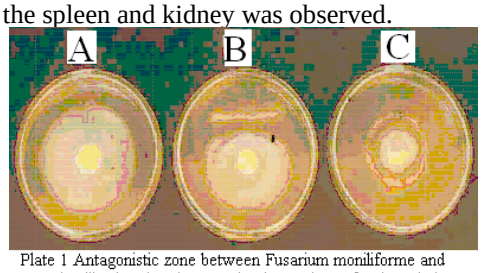

Plate 1 Antagonistic zone between Fusarium moniliforme and Lactobacillus brevis. Photograph taken 5 days after inoculation. A = control; B = Fokkema/conventional streak method; C = concentric ring bioassay . X3

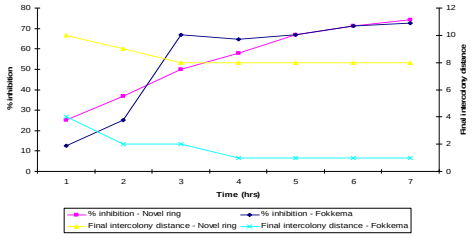

Fig. 1: Effect of antagonistic ability of *Lactobacillus brevis* on Fusarium moniliforme inn terms of intercolony distance and percentage inhibition of radial growth rate.

Table 1: Effect of the administration of *Fusarium moniliforme and Lactobacillus brevis* on biochemical indices of albino rat plasma

Treatment	AST (IU/l)	ALP (IU/L)	ALT (IU/L)	Albumin (g/dL)	Globulin (g/dL)	Protein (g/dL)
Basal diet alone (Control)	$9.42^{a} \pm 2.28$	$21.09^{a} \pm 3.89$	$3.38^{a} \pm 0.96$	$11.70^{a} \pm 0.82$	$29.93^{a} \pm 4.75$	$47.41^{a} \pm 9.04$
Basal diet + fungus Alone (A)	$11.11^{b} \pm 1.57$	$28.13^{c} \pm 5.60$	$5.79^{c} \pm 2.08$	$11.60^{a} \pm 0.16$	$30.25^{abc} \pm 9.49$	$28.42^{c} \pm 17.84$
Basal diet + fungus And bacterium (B)	$9.66^{ab} \pm 0.96$	44.53 ± 2.44	$5.43^{b} \pm 0.46$	$12.36^{b} \pm 2.63$	$20.01^{b} \pm 6.02$	$33.22^{ab} \pm 9.59$
Basal diet + Bacterium alone (C)	$8.33^{a} \pm 3.26$	$40.48^{d} \pm 7.64$	$5.31^{b} \pm 0.97$	$12.37^{b} \pm 4.57$	$29.96^{a} \pm 5.63$	$38.82^{ab} \pm 7.71$

AST, Aspartate aminotransferase; ALP, Alkakine phosphatase; ALT, Alanine aminotransferase.
Values are mean ± S.E. (n=4)
Means with the same superscript letter(s) along the same column are not significantly different (P>0.05)

Table 2 Effect of administration of *Fusarium moniliforme and Lactobacillus brevis* on the haematological parameters of albino rats.

Treatment	PCV %	Hb g/L	WBC $X10^{9}/L$	Neutrophils %	Lymphocyte %	Monocytes %	Eosinophils %
Basal diet alone (Control) BUU	$47.25 \pm 2.06_{a}$	$16.00 \pm 0.81_{a}$	$7.05 \pm 2.75_{a}$	$55.75 \pm 6.34_{a}$	$42.25 \pm 7.27_{a}$	$50 \pm 0.57_{a}$	0.00 ± 0.00
Basal diet+ fungus Alone (A) BIU	$49.25 \pm 1.50_{a}$	$16.75 \pm 0.50_{a}$	$6.45 \pm 4.37_{a}$	$59.00 \pm 7.07_{abc}$	$40.25 \pm 7.13_{ab}$	$1.25 \pm 0.50_{a}$	$2.00 \pm 0.00_{ab}$
Basal diet + fungus And bacterium (B) BUT	$52.25 \pm 1.50_{ab}$	$17.75 \pm 0.50_{ab}$	$7.37 \pm 2.69_{a}$	$62.00 \pm 2.58_{b}$	$37.00 \pm 1.63_{b}$	$2.75 \pm 0.95_{ab}$	$0.00 \pm 0.00_{a}$
Basal diet + bacterium Alone (C) BIT	$50.00 \pm 0.00_{a}$	$17.00 \pm 0.00_{a}$	$7.22 \pm 2.71_{ab}$	$59.00 \pm 7.62_{ab}$	$40.75 \pm 7.18_{a}$	0.00 ± 0.00	$1.50 \pm 0.57_{a}$

PCV, Packed Cell Volume; Hb, Haemoglobin; WBC, White Blood Cell
Values are mean of four replicates ± standard deviation
Values followed by similar alphabets along the same column are not significantly different (P<0.05)

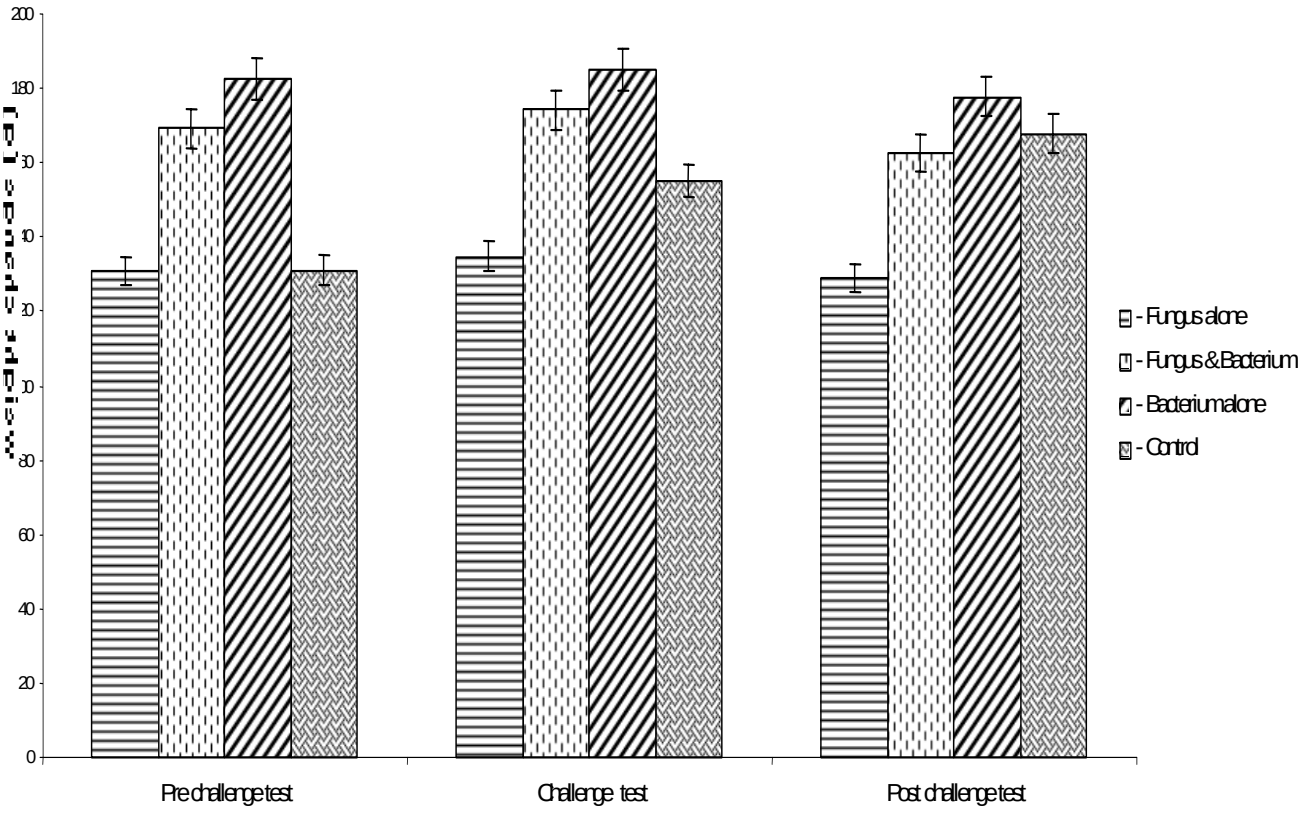

Fig 2: Periodic weight changes of rats dosed with *Fusarium moniliforme* and *Lactobacillus brevis*

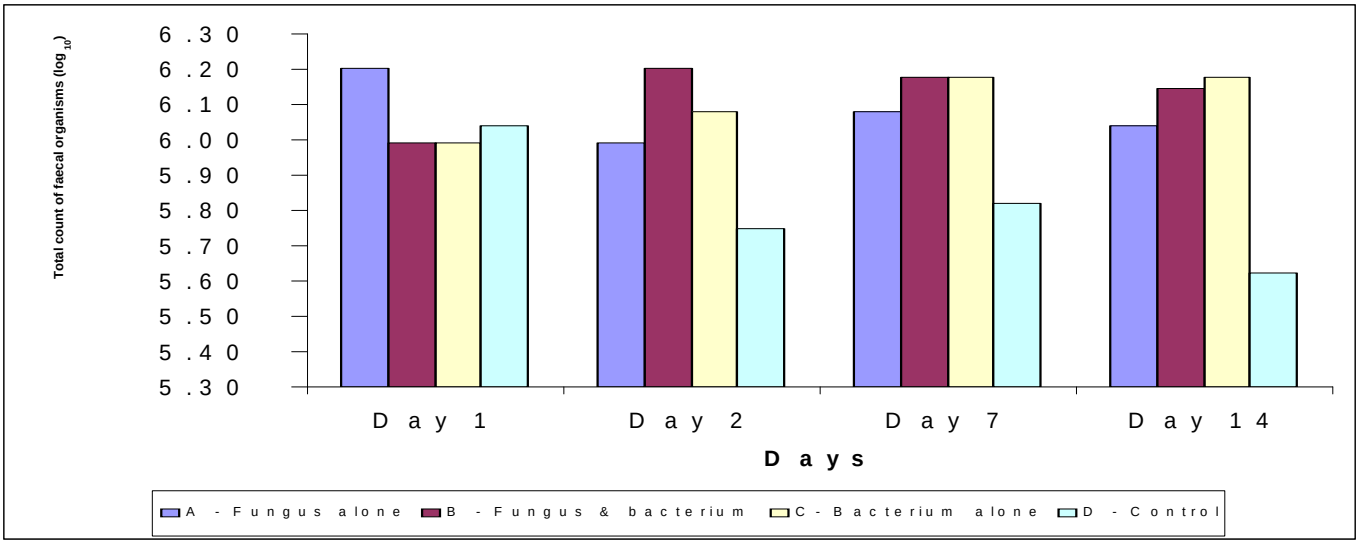

Fig. 3: **Total count of faecal organisms in rats dosed *with Fusarium moniliforme* and *Lactobacillus brevis***

CONCLUSION

L. brevis has a stimulatory effect on humeral immunity of albino Wistar rats with the following benefits: antimicrobial activity against important pathogens and a food spoilage fungus: *Fusarium moniliforme:* hepatoprotective effect as a result of the ability to lower plasma aminotransferase levels and immunostimulatory effect.

Correspondence to:
Agarry Olubunmi Olaitan
Department of Biological Sciences,
University of Abuja, Nigeria
Telephone: +234-807-8160565
Email: oluagarry@yahoo.com

REFERENCES

1. Aattouri., N,, Bouras, M., Tome, D., Marcos, A. and Lemonnier, D. (2001) Oral Ingestion of lactic acid bacteria by rats increases lymphocyte proliferation and interferon a production. *British Journal* of *Nutrition 87*, 367-373
2. Adetuyi ,F.C., Cartwright, O.W (1985) Studies on the antagonistic activity of bacteria endemic to cereal seeds. I Quantification of antagonoists activity. *Annals*.of *Applied Biology 107*, 33-43
3. Agarry, O.O. (2006) Effect of microbial antangonists on the Haematological Responses of Albino Rats *Journal of Food Technology 4(3)*: 371-375
4. Agarry, O. O. and Osho, B. I. (2005) *In vitro* and *in vivo* inhibition of *Aspergillus fumigatus* by *Pseudomonas fluorescens* used as a microbial antagonist. *Pakistan Journal of Nutrition* 4 (6) 371-375
5. Akihiko, U. (1994). *The amazing Lactobacillus brevis*, Shiki Shuppan Co., Limited.
6. Akihiko, U. (1995). *The secrets of Lactobacillus brevis*, Shiki Shuppan Co., Limited.
7. Aletor, V.A. (1993) Cyanide in *gari*. Assessment of some aspects of the nutrition, biochemistry and haematology of the rats fed *gari* containing varying residual cyanide levels. *International Journal of Food Sciences and Nutrition 44*, 289-292
8. American Liver Foundation (1995) *Liver function tests.* Available online at: http://www.gastro.com/liverpg/ifts.htm
9. American Liver Foundation (1997) Liver function tests. Available online at: http://www.in.ucsf.edu/ALF/info/infoliverfx.html
10. Anaissie, E., Kantarijian, H., Ro, J., Hopfer, R., Rolston, K., Fainstein, V. and Bodey, J. (1988). The emerging role of Fusarium infections in patients with cancer. *Medicine (Baltimore). 67:*79-83.
11. Aning, K.G, Ologun, A.G., Onifade, A., Alokan, J,A., Adekola .A,I., Aletor, V.A. (1998) Effects of replacing dried brewer's grains with sorghum rootlets on growth, nutrient utilization and some blood constitutions in the rat. *Animal Feed Science Technology 71*, 185-190
12. Baker, H.J., Lindsey, J.R., Weisbroth, S.H. (1979) *The Laboratory Rat. (Vol 1) Biology and Diseases*, Academic Press, New York, X pp
13. Baker, F.J., Silver, R.E (1985) *Introduction to Medical Laboratory Technology* (6^{th} Edn), Macmillan Press, City, pp 320-328
14. Baron, D.N., Whicher, J.T., Lee, K.E. (1994) *A nee short textbook of chemistry pathology*, ELBS, 5^{TH} EDITION. PP. 151-130.
15. Bertazonni, M.E., Benini, A.. Marzotto, M, Hendriks, H., Sbartbati, A.and Dellaglio, F. (2001). Preliminary screening of health-promoting properties of new *Lactobacillus strain: in vitro and in vivo.* HEALFO abstracts Italy.
16. Casas, I.A. and Dobrogosz, W.J. (2000). Validation of the probiotic concept: Lactobacillus *reuteri* confers broad spectrum protection against disease in humans and animals. *Microbial Ecology. Health Diseases. 12*:247-285.
17. Chang, Y., Kim, J., Kim, W. and Park, Y. (2001). Selection of a potential probiotic *Lactobacillus* strain and subsequent *in vivo* studies. *Antonie Van Leeuwenhoek*. pp 193-199.
18. David, E., Johnston. M,D. (1999) Special considerations in interpreting liver Function tests. *American Family Physician*. Available online at: *http://www.aafp.org/afpagoer5ap/html*.
19. Deshpande, S.D. and Koppika, G.V. (1999). A study of mycotic keratitis in Mumbai. *Indian Journal Pathology Microbiology. 42*:81-7.
20. Fokkema, N.J. (1973) The role of ssaprophytic fungi in antagonism against *Drechslera sorokianiana* (*Helminthosporium sativum*) on agar plates and rye leaves with pollen. *Physiology.Plant pathology 3*, 15-105.
21. Fuller, R. (1992). *Probiotic: The scientific basis.* Ed. R. Fuller. London: Chapman and Hall.
22. 22. Guarro,J. and Gene, J. (1995). Opportunistic fusarial infections in humans. *European Journal Clinical Microbiology Information D. 14*:741-754.
23. Henriksson, A, and Conway, P.L. (2001 Isolation of human faaaecal *Bifidobacteria* which reduces signs of *Salmonella* infection when orogastrically dosed to mice. *Journal of Applied Microbiology 90*, 223-228
24. Heyman, M. (2000) Effect of Lactic Acid Bacteria on Diarrheal Diseases. *Journal of the American College of Nutrition* Vol. 19 No. 90002, 137S – 146S.
25. Johnston, D.E. (1999) Special consideration in interoreting liver function tests. *The American*

Academy of Family Physicians April 15, Vol. 59(8)
26. Liver Function Tests (2005) Lab Tests Online [uk] labtestsonlineuk@acb.org.uk
27. Mitruka, B.M.,, Rawnsley, H.M. (1977). Clinical Biochemical and Haematological Reference Values in Normal Experimental Animals, Masson. New York
28. Mokady, I.C., Abramovici, A,, Cogan, U. (1989) The valuation of *Dunaviela bardawilla* as a potential food supplement. *Food Chem*istry *Toxicology 27*, 221-226
29. Monica, C. (2000) *District Laboratory Practice in Tropical countries: Part 2*, Low Price Edition Cambridge University Press. United Kingdom, pp 108, 112 and 326.
30. Oboh, G., Akindahunsi, A.A. and Oshodi, A.A. (2000). Aflatoxin and moisture content of microfungi fermented cassava products (flour and gari). *Applied Tropical Agriculture. 5(2):* 154 – 157.
31. Silva, A.M,, Bambina, E.A., Oliverira, A.L., Souza, P.P., Gomes, D.A., Viera, E.C and Nicoli, J.R, (1999). Protective effect of bifidus milk on the experimental infection with *Salmonella enteridis* subsp. *Typhimirium* in conventional and gnobiotic mice. *Journal of Applied Microbiology, 86*:331-336.
32. Tanure, M. A., Cohen, E,J,, Sdeshk S,, Rapuano, C.J.and Laibson,P,R, (2000). Spectrum of fungal keritits at Wills Eye Hospital, Philadelphia, Pennyslaavnia. Cornea. 19: 307 12
33. Weihe ,W.H. (1987). *The laboratory rat. In T. pool (ed). UFAW handbook on the care management of laboratory animals.* 6th ed Longman Scientific and Technical, Harlow UK, Williams and Wilkins Co. Baltimore, MD.
34. Younossi , Z.M.and Mehta. N. (1998) Evaluating asymptomatic patients with mildly elevated liver enzymes. *Cleveland Clinic Journal of Medicine*: onine continuing Medical E ducation 65(3) 27p, http:www.ccf.org/ed/online4/art498.httm
35. Yoshindo (2005). Health foods – Related information (1) The power of Lactobacillus brevis. *http://www.yosindo.cojp/kenko2e.html*
36. Oboh, G. and Akindahunsi, A.A. (2004) Nutritional and toxicological evaluation of *Saccharomyces cerevisiae* fermented cassavaa flour. *Journal of Food Composition and Analysis* 1-8.
37. Oyetayo, V.O. (2006). Untapped health promoting potential of nitrogenous cereal-based African fermented foods and beverages. *European Journal of Nutraceuticals and Functional Foods. AgroFOOD Industry hi-tech. Anno 17-No 3:*36-37.
38. Pitt, J.I. (2000). Toxigenic fungi; which are important. *Medical Mycology 38*:17-22.
39. Prescott, L.M., Harley, J.P., Klein, D.A. (1999). *Microbiology;* 5th Edn. The McGraw-Hill Companies, Inc. USA, pp:704-733 and 818-819.
40. Schell, W.A. (2000). Histopathology of fungal rhinosinusitis. Orolaryngol Clinical *Nutrition America 33:* 251 –276. VII, VIII, NIL 5.

7/27/2010