Journal of Food Technology 4 (2) : 152-155, 2006

Toxicological Evaluation of *Bacillus* Species Associated with Cassava Products

O.O. Agarry
Department of Microbiology, Federal University of Technology
P.M.B. 704, Akure, Nigeria

Abstract: In the present study pure strains of *Bacillus subtilis*, *B. pumilus* and B. *sphaericus* were used to antagonized indigenous fungi of cassava products with the aim of evaluating their potentials in reducing the toxicological and pathological consequences associated with the indigenous fungi. The protective effect was studied using rat bioassay. Toxicological data of rat plasma showed that *Bacillus* species had liver improvement functions. There was a significant ($p = 0.05$) decrease in the plasma aspartate aminotransferase of treated rats indicating no possible damage to the liver (hepatotoxic), while there was non significant ($p = 0.05$) rise in the plasma protein. Moreover, the administration of *Bacillus* sp. had no negative haematological (packed cell volume, haemoglobin count and white blood cell counts) effect. Further pathological investigation revealed that the kidney and pancreas were protected by the administration of the organism.

Key words: Grated cassava, starch, flour, *Bacillus* sp., toxicology

INTRODUCTION

Cassava (*Manihot esculenta*, Crantz) is affected by fungi, bacteria and mycoplasma agent [1]. It is now considered that disease are among the major limiting factors to the fortress of the development and bulk yield of cassava. The color, taste, texture and consistency of processed cassava food products have being the major area of consideration among the consumers but little consideration is made on microbial quality of cassava products. The inside of a newly harvested plant usually contains no microbes, but this may soon change from effects of processing. Some of the microbes are introduced into the food substance from outside surface of the food while others come from equipment and other human sources such as contaminated water during processing. Several types of microorganisms have been known to affect the quality of food, thereby constituting health hazards when foods contaminated with these organisms.

Cassava products lose their quality by the action of bacteria and fungi. Most of the bacteria and fungi isolated from analyzed cassava products are common inhabitants of soil, water, air and the body of humans and animals. *Aspergillus flavus* and *A. niger* were among the mould species isolated from cassava tubers[2, 3]also reported their occurrence in 'gari', a product of cassava tuber. These species together with *A. chevalier*, *Syncephalastrum racemosum* and *Penicillium citrinum* were isolated from gari during storage in polythene and Hessian bags [4]. The presence of moulds in any agricultural product is usually undesirable. The role of most of the fungi found in the cassava products in the production of certain toxins generally called 'mycotoxins' have been established[5, 6]. The aerobic spore-forming bacteria have been enumerated and used to investigate quality of cassava flour. These organisms have been implicated in spoilage of bread and starch-based foods and in food intoxication [7]. The production of antifungal antibiotics confirmed the possibilities of bio competitive exclusion of pathogenic organisms from food. Many species of *Bacillus* are known to suppress fungal growth *in vitro* by the production of one or more antifungal antibiotics[8, 9] also reported on *In vivo* suppression of fungal growth by some antibiotic producing strains of *Bacillus*. These antibiotics produced *in vitro* however are generally assumed to be the compound responsible for bio control *In vivo*. The potential of *B. subtilis* as a biological control agent against fungal pathogen has been reported[9,10 11]. Some representatives of the genus such as *B subtilis* and *B. licheniformis* are Generally Recognized As Safe (GRAS) bacteria[12]. *Bacillus* species are a group of bacteriocinogenic microorganism important in alkaline- fermented foods and beverages[13, 14]. This study therefore sought to investigate the potential inhibitory effects of *Bacillus* species isolated from grated cassava, starch and flour. Furthermore, *Bacillus* species administered *in vivo* were evaluated for their ability to reduce the toxicological and pathological consequences associated with indigenous fungi experimentally used to infect rats.

MATERIALS AND METHODS

Cassava tubers were collected within Akure metropolis, Nigeria and processed into grated

cassava, starch and flour using the methods of Onabolu *et al.*(1998). The microorganisms used in this investigation (*Aspergillus niger*, *Fusarium nivale*, *Penicillium chrysogenum*, *Bacillus subtilis*, *B. pumilus* and *B. sphaericus)* were isolated from the products. Large numbers of bacteria cells were prepared and preserved by lyophilization. Prior to the *in vivo* feeding trial, the bacterial cells were reconstituted and the recovery rate assessed by viable cultures. Fungi cells were harvested by washing with sterile water into suitable receptacles. The cell suspension was diluted and the cells counted using Hawskley haemocytometer with Neubauer ruling.

Bioassays: The toxicological quality was determined using Wistar albino rats divided into three groups. Each of the animals in each group was housed in individual wire cages in a room maintained at room temperature. Animals were subjected to three treatments using the modified method of[15]; fungus-infected and bacterium-treated rats + basal diet (group A); uninfected animals given only bacterium + basal diet(group B); appropriate control group (C) of uninfected, untreated rats fed with basal diet only Animals were orogastrically dosed with 0.3mL^{-1} each of 10^8 spore mL of the fungus and 10^6 cfu g of the bacterium, respectively. The treatments were repeated the second day. A post-ingestion period of 14 days was observed after the administration of the cultures.

Haematology and blood chemistry tests: The haematological tests namely Packed Cell Volume (PCV), While Blood Cell (WBC) counts, Haemoglobin (Hb) counts, lymphocytes, monocytes, neutrophils and eosinophils counts were conducted according to the conventional methods reported by[16]. The biochemical tests namely albumin, globulin, protein, Aspartate aminontransferease (AST), Alanine aminotransferease (ALT) and Alkaline Phosphatase (ALP) were determined by the conventional methods reported by Aning[17].

Histopathological tests: At autopsy the internal organs were inspected for morphological lesions. For the histopathological study, samples of the following organs: Pancreas, liver, kidney, stomach and small intestine from each animal were fixed in buffered 10% formalin-saline. After dehydration and embedding in paraffin wax, 5µm sections were cut and stained for examination in the microscope.

Analysis of data: The results were pooled and expressed as mean ± standard deviation. The data were analysed by one way analysis of variance (ANOVA) SPSS 10.0.

RESULTS AND DISCUSSION

The species of *Bacillus* used in this study inhibited the growth of *Aspergillus niger, Fusarium moniliforme* and *Penicillium chrysogenum as* reported by Mokady[18]. The relationship between diet and disease can be ascertained by biomarkers since they provide a link between the consumption of specific foods and biological outcome [19]. Major biomarkers such as plasma enzymes changes in disease are related in many ways to cell pathology[20]. There was a significant difference (p = 0.05) between the AST of the control and that of the *Bacillus* species administered as microbial antagonists (Table 1). The rise in AST in rats fed with *Aspergillus niger* and *Fusarium nivale* could be attributed to possible secretion of mycotoxins. Increase in blood AST values may be attributed to both liver damage and possible damage to the heart[21]. Reduction of AST values was observed in rats administered *Bacillus* species separately; showing that the bacteria reduced the infection on the host. This result implies that the organisms possess antimycotic properties capable of reducing the severity of attack of the pathogen on the host. In chronic liver diseases such as Hepatitis C and cirrhosis, the ALT correlate moderately well with liver inflammation[22]. In affected liver, both the ALT and ALP levels are increased[23]. Mild elevation of the ALP laws in the blood of rats treated with *Bacillus subtilis* and *B. sphaericus* are non-specific and may be caused by a wide range of liver diseases[24, 25]. No significant change (p = 0.05) in plasma ALT contents of albino rats fed with *Bacillus* spp. compared with that of the control diet. The implication of these results is that, there is no pronounced toxicological effect in rats treated with these organisms. Previous studies have demonstrated that some strains of *Bacillus* have the ability to suppress the growth of fungi *In vivo*[9, 10].

Changes in plasma proteins occur in chronic hepatitis and cirrhosis, due to the prolonged or extensive liver cell impairment. The obtained plasma protein levels were significantly not different (p = 0.05) to the control. The result indicate d that the *Bacillus* isolates administered to the rats are not likely to cause liver damages. Furthermore, significant improvement was observed in the Packed Cell Volume (PCV) and Haemoglobin (Hb) of rats administered the bacteria treatment compared to the control. The WBC counts were however significantly lower for *B. pumilus* and *B. sphaericus* treated rats. The low WBC count is an added advantage since increased numbers are associated with infections and leukamias. The basophils neutrophils, eosinophils and lymphocytes in rats maintained on the treated diets were significantly different from the control (Table 2). The results compared

Table 1: Plasma chemistry evaluation of albino rats administered microorganisms, *Bacillus* Sp., as antagonists

Parameters	Control	*Aspergillus niger* and *B. subtilis*	*Bacillus subtilis*	*Fusarium nivale* and *B. pumilus*	*B. pumilus*	*Penicillium chrysogenum* and *B. sphaericus*	*B sphaericus*
AST (IU/L)	$9.42^{a}\pm2.281$	$1.1T^{ab}\pm1.57$	$8.57^{a}\pm2.06$	$20.77^{b}\pm20.69$	$9.78^{a}\pm5.38$	$8.69^{ab}\pm3.20$	$4.46^{c}\pm1.38$
ALP (IU/L)	$21.09^{A}\pm3.89$	$28.77^{ab}\pm3.64$	$21.31^{b}\pm2.51$	$29.40^{ab}\pm5.63$	$28.97^{c}\pm3.87$	$32.60^{b}\pm2.24$	$24.93^{bc}\pm4.47$
ALT(IU/L)	$3.38^{a}\pm0.96$	$5.43^{b}\pm2.06$	$3.38^{b}\pm1.24$	$2.41^{b}\pm1.26$	$4.46^{a}\pm3.96$	$2.17^{a}\pm1.44$	$2.77^{a}\pm1.49$
Albumin (g/dL)	$11.70^{a}\pm0.82$	$12.37^{a}\pm0.10$	$12.36^{b}\pm0.03$	$12.40^{a}\pm0.05$	$12.00^{b}\pm0.17$	$12.52^{a}\pm0.48$	$11.27^{b}\pm0.00$
Globulin (g/dL)	$29.93^{a}\pm4.75$	$53.36^{c}\pm1.80$	$46.99^{b}\pm7.32$	$25.48^{bc}\pm3.95$	$27.45^{a}\pm2.31$	$18.15^{a}\pm5.17$	$35.25^{a}\pm2.50$
Total Protein (g/dL)	$47.41^{a}\pm9.04$	$57.42^{b}\pm15.20$	$45.33^{ab}\pm13.06$	$40.28^{a}\pm5.75$	$4.24^{ab}\pm6.24$	$34.31^{a}\pm4.71$	$41.90^{ab}\pm1.81$

Values are mean of four replicates ± standard deviation. Means followed by similar superscript letter(s) along same row are not significantly different at 0.05

Table 2: Heamatological Evaluation of Albino Rats Administered Basal Diet with Microorganisms

Parameters	Control	*Aspergillus niger* and *B. subtilis*	*Bacillus subtilis*	*Fusarium nivale* and *B. pumilus*	*B. pumilus*	*Penicillium chrysogenum* and *B. sphaericus*	*B sphaericus*
PCV (%)	$47.25^{a}\pm2.06^{a}$	48.00 ± 3.46^{a}	52.50 ± 5.56^{ab}	54.00 ± 2.44^{b}	50.00 ± 2.44^{ab}	53.75 ± 2.87^{b}	55.50 ± 3.31^{ab}
Hb(g/L)	16.00 ± 0.81^{a}	16.25 ± 1.25^{a}	17.75 ± 1.70^{abc}	18.25 ± 1.25^{b}	17.00 ± 0.81^{a}	18.25 ± 0.95^{b}	18.75 ± 0.95^{bc}
WBC(10^{9}/L)	7.05 ± 2.75^{a}	6.37 ± 2.98^{a}	8.97 ± 3.98^{b}	5.25 ± 1.33^{ab}	6.87 ± 1.65^{ab}	5.07 ± 2.01^{a}	5.95 ± 1.97^{ab}
Neutrophils (%)	55.75 ± 6.34^{a}	50.75 ± 5.91^{ab}	46.75 ± 12.95^{ab}	43.50 ± 13.77^{ab}	52.00 ± 13.11^{ab}	55.00 ± 13.68^{ab}	60.75 ± 9.42^{b}
Lymphocytes(%)	42.25 ± 7.27^{a}	48.25 ± 6.23^{a}	52.00 ± 12.83^{ab}	54.75 ± 13.02^{a}	46.00 ± 15.01^{ab}	44.50 ± 14.24^{a}	37.25 ± 7.36^{a}
Monocytes(%)	2.50 ± 0.57^{a}	1.50 ± 0.57^{a}	0.00 ± 0.00	3.00 ± 1.41^{ab}	3.00 ± 1.41^{b}	0.00 ± 0.00	3.75 ± 1.50^{ab}
Eosinophils (%)	0.00 ± 0.00	1.75 ± 0.50^{a}	3.50 ± 1.73^{b}	0.00 ± 0.00	1.75 ± 0.50^{a}	2.50 ± 1.00^{ab}	1.75 ± 0.95^{a}

Values are mean ± S.D (n = 4) Values followed with similar alphabets along same row are not significantly different ($p<0.05$)

favourably with the results reported on albino rats fed sorghum and brewer's grains[16] and albino rats fed *Saccharomyces cerevisiae* fermented cassava flour diet[6]. The improvement in blood composition followed by the treatment indicated an immunological security for the group of animals given such treatment.

The result of the histological analysis confirmed that the administration of *Bacillus* sp. did not cause any damage to the kidney and pancreas. However, it causes disorientation of the muscular layer of the stomach and small intestine. The pathological studies had reveled that metabolites produced from *Bacillus* sp. initiated a broad range of biological effects by protecting the internal organs of the animals, liver function (enzyme activity) and haematological improvement.

REFERENCES

1. IITA Research Manual, 1990. Cassava in Tropical Africa: A Reference Manual. IITA Ibadan, Nigeria, pp: 16-113.
2. Ekundayo, J.A. and T.M. Daniel, 1973. Cassava rot and its control. Trans Br. Mycol. Soc., 61: 27-32.
3. Adeniyi, M.O., 1976. Fungi associated with the deterioration of 'gari'. Nigerian J. of Plant Prot., 2: 74-77.
4. Oyeniran, J.O., 1981. Mould development in gari during storage in polythene and Hessian bags Rep. Nigeria Stored prod. Res. Inst. (1978/79) Tech. Rep., 11: 93-99.
5. Sasson, A., 1988. Fermentation in a solid medium, Biotechnologies and Development, pp: 79-120.
6. Vanneste, G., 1982. Enrichissement proteinique du manix par fermentation forgique. Rev. Fermentation industries Alimentaires, 37: 19-24.
7. Oboh, G., A.A. Akindahunsi and A.A. Oshodi, 2000. Aflatoxin and moisture content of micro-fungi fermented cassava product (flour and gari). Applied Trop. Agric., 5: 154-157.
8. Jay, J.M., 1978. Modern food Microbiology (2nd Edn.). D. Van Nostand Company. New York.
9. Katz, E. and A.L. Demain, 1977. The peptides antibiotics of Bacillus chemistry, biogenesis and possible functions. Bacteriological Rev., 41: 449-474.
10. Fravel, D.R., 1988. Role of antibiosis in the biocontrol of plant disease. Ann. Rev. Phytopathol., 26: 75-91.
11. Fereira, J.H.S., F.N. Matther and A.C.Thomas, 1991. Biological control of *Eutypalata* on grapevine by an antagonistic strain of *Bacillus subtilis*. Phytopathol., 81: 283-287.
12. Shenin, I.D., L.F. Kruglikova, G.N. Kal'ko and I.I. Novikova, 1995. The characteristics of A alirin B-1- the basic component of a fungicidal preparation produced by the Bacillus subtilis 10- VIZR strain. Antibiotikii Klimioterapiya, 151: 159-165.
13. Sharp, R. J., M.D. Scawen and T.Alkinson, 1989. Fermentation and Downstream Processing of *Bacillus. In Bacillus,* Edn. Harwood, C.R. New York: Plenum press, pp: 255-292.
14. Odunfa, S.A. and G.F. Oyeyiola, 1985. Microbiological study of fermentation of *Igba* a Nigerian indigenous fermented food flavour. J. Plant Food 6: 155-163.
15. Wang, J. and D.Y.C. Fung, 1996. Alkaline-fermented foods: A review with emphasis on pidam fermentation, Critical Rev. Microbiol., 22: 101-138.

16. Henriksson, A. and P.L. Conway, 2001. Isolation of human faecal *Bfidobacteria* which reduces signs of *Salmonella* infection when orogastrically dosed to mice J. Applied Microbiol., 90: 223-228.
17. Aning, K.G., A.G. Ologun, A.Onifade, J.A. Alokan, A.I. Adekola and V.A. Aletor, 1998. Effects of replacing dried Brewer's grains with sorghum rootlets on growth, nutrient utilization and some blood constituents in the rat. Ani. Feed Sci. Technol., 71: 185-190.
18. Mokady, I.C, A. Abramovici and U. Cogan, 1989. The evaluation of *Dunaviela bardawilla as* a potential food supplement Fd. Chem. Toxic, 27: 221-226.
19. Agarry, O.O., F.A. Akinyosoye and F.C. Adetuyi, 2005. Antagonistic properties of micro organisms associated with cassava (*Manihot esculenta*, Crantz) products. African J. Biotechnol., 4: 627-632.
20. Branca, F., A.B. Hawley, B. Pool-Zobel and H. Verhagen, 2001. Biomarkers of exposure and effect in relation to quality of life and human risk assessment. British J. Nutr., pp: 55-92.
21. Baron, D.N., J.T. Whicher and K.E. Lee, 1994. A New Short Textbook of Chemistry Pathology, ELBS, (5th Edn.). pp: 151-156.
22. David, E. and M.D. Johnston, 1999. Special considerations in interpreting liver function tests. American family physician http//www.aafp.org/ afpagoer 5ap/html.
23. Schmidt, G. and F. Schmidt, 1976. Brief Guide to Practical Enzyme Diagnosis, (2nd Edn.). Buehringer Gmbit, pp: 108-123.
24. Strove, E.A. 1989. Biochemistry. Mir publisher (1st Edn.). pp: 425-432.
25. American Liver Foundation, 1995. Liver function tests, http://www.gastro.com/liverpg/fts.htm.
26. American Liver Foundation 1997. Liver function tests, http://www. in ucsf.edu/ALF/ info/ infoliverfx.html.