Journal of Food Technology 4(3): 174-177, 2006

Effects of Microbial Antagonists on the Haematological Response of Albino Rats

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

Abstract: A total of 7 bacteria, which displayed antagonist activity against indigenous fungi *in vitro*, were isolated from grated cassava, starch and flour samples. Cultures were pooled and tested for anti-fungi activity, inhibited growth of the fungal pathogens and the protective effects of the various bacterial isolates. The effects of the treatment on the haematological factors were studied on albino rats. No negative haematological (packed cell volume, haemoglobin counts, white blood cell counts and differential white cell counts) effect was observed. However, treatments with *Bacillus* sp., *Pseudomonas fluorescens* and *Escherichia coli* resulted in low WBC counts when compared with the control. The RBC and WBC morphology for all appeared normal. The improvement in blood composition that followed treatment of the animals with the bacterial isolates indicated an immunological security.

Key words: Blood cell indices, microbial antagonist, wistar albino rats

INTRODUCTION

The concept of developing alternatives to the mass use of chemicals for disease control has generated other approaches to crop protection. One of these is based on the principle of microbial antagonism. The mechanistic basis of biological control include antibiosis which is an inhibitory effect one organism exerts upon another organism through the production of antibiotic compounds. Other antimicrobial substances of microbes with antagonistic properties include biocides, probiotics, organic acids, hydrogen peroxide and carbon dioxide etc. The uses of these substances produced by bacteria to inhibit the growth of other microbes were reported by several authors[1-6]. Recently, Agarry[6] reported that antagonism of fungal pathogens by bacterial isolates both of cassava products origin is as a result of the active production by bacteria of diffusible antimicrobial compounds. The inhibitory activities due to bacteriocins and organic acid production. In view of the fact that most microbial activities are usually accompanied by secretion of some harmful metabolites[7], the safety of these specific antagonism between the bacteria and fungi in the body system is to be determined. This study therefore sought to investigate the effects of bacteria treatments on the haematological parameters of albino rats.

MATERIALS AND METHODS

Test organisms: Seven different fungal isolates namely: *Aspergillus niger*, *Fusarium moniliforme*, *A. fumigatus*, *F. nivale*, *Syncephalastrum racemosum*, *Rhizoctonia* sp. and *Penicillium chrysogenum* were antagonized with seven bacterial isolates namely*:* *Bacillus subtilis*, *Lactobacillus brevis*, *Pseudomonas fluorescens*, *B.pumilus*, *Escherichia coli*, *Staphylococcus aureus* and *B. sphaericus*, all isolated from grated cassava, starch and flour as previously described[8]. The fungi were cultivated on Sabouraud Dextrose Agar (SDA) (Oxoid Ltd., London) for 7 days at 25°C. The cells were separately harvested by washing with sterile water into suitable receptacles. The cell suspension was then diluted with the same liquid and the spores counted using a Hawksley haemocytometer with Neubauer ruling. Large numbers of the bacterial isolates cells were prepared by incubating isolates in MacConkey, (MCA), Nutrient (NA) and de Mann Rogosa Sharpe (MRS) broth respectively at 37°C for 48 h[9]. The cells were harvested by centrifugation at 10000 g for 15 min, washed twice with sterile water and collected by centrifugation (10000g for 15 min). The washed cells were re-suspended in rehydrated skim milk (10% w/v), Iyophilized and stored at -20°C until use. The concentration of viable cells in the final powder was determined by serial dilution and subsequent plating on NA, MCA and MRS plates.

Animals: Wistar albino rats (6-8 and 12-14 weeks) were used in these experiments. The animals were housed in standard cages with food and water *ad libitum*, at room temperature (25±2°C) with artificial light. The animals kept under controlled environment following the standard operating procedures of the animal house facility.

Table 1: Effect of administration of *A. niger* and *B. subtilis* on the haematological parameters of albino rats

Treatment	PCV (%)	Hb g L^{-1}	WBC $X10^9$/L	Neutrophils (%)	Lymphocytes (%)	Monocytes(%)	Eosinophils (%)	MCHC(%)
Control	$47.25\pm2.06_b$	$16.00\pm0.81_a$	$7.05\pm2.75_a$	$55.75\pm6.34_a$	$42.25\pm7.27_a$	$2.50\pm0.57_a$	0.00 ± 0.00	33.86
(A)	$50.25\pm3.77_a$	$17.00\pm1.41_a$	$6.42\pm2.59_a$	$64.50\pm4.70_c$	$34.75\pm5.61_a$	$2.00\pm0.00_a$	$1.25\pm0.50_a$	33.83
(B)	$48.00\pm3.46_a$	$16.25\pm1.25_a$	$6.37\pm2.98_a$	$50.75\pm5.91_{ab}$	$48.25\pm6.23_a$	$1.50\pm0.57_a$	$1.75\pm0.50_a$	33.85
(C)	$52.50\pm5.56_b$	$17.75\pm1.70_{abc}$	$8.97\pm3.98_b$	$46.75\pm12.95_{ab}$	$52.00\pm12.83_{ab}$	0.00 ± 0.00	$3.50\pm1.73_b$	33.81

Control: Basal diet only; A: Basal diet + fungus only; B: Basal diet + fungus and bacterium; C: basal + bacterium only PCV, Packed Cell Volume; Hb, Haemoglobin; WBC, White Blood Cell Values are mean of four replicates±standard deviation Values followed by similar alphabets (a,b,and c) 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^{-1}	WBC $X10^9$/L	Neutrophils (%)	Lymphocytes (%)	Monocytes (%)	Eosinophils (%)	MCHC(%)
(Control)	$47.25\pm2.06_a$	$16.00\pm0.81_a$	$7.05\pm2.75_a$	$55.75\pm6.34_a$	$42.25\pm7.27_a$	$2.50\pm0.57_a$	0.00 ± 0.00	33.86
(A)	$49.25\pm1.50_a$	$16.75\pm0.50_a$	$6.45\pm4.37_a$	$59.00\pm7.07_{abc}$	$40.25\pm7.13_{ab}$	$1.25\pm0.50_a$	$2.00\pm0.00_{ab}$	34.01
(B)	$52.25\pm1.50_{ab}$	$17.75\pm0.50_{ab}$	$7.37\pm2.69_a$	$62.00\pm2.58_b$	$37.00\pm1.63_a$	$2.75\pm0.95_{ab}$	$0.00\pm0.00_a$	33.97
(C)	$50.00\pm0.00_a$	$17.00\pm0.00_a$	$7.22\pm2.71_{ab}$	$59.00\pm7.62_{ab}$	$40.75\pm7.18_a$	0.00 ± 0.00	$1.50\pm0.57_a$	34.00

Control: Basal diet only; A: Basal diet + fungus only; B: Basal diet + fungus and bacterium; C: basal + bacterium only, 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 ($p<0.05$)

Table 3: Effect of administration of *Aspergillus fumigatus* and *Pseudomonas fluorescens* on the haematological parameters of albino rats

Treatment	PCV (%)	Hb g L^{-1}	WBC $X10^9$/L	Neutrophils (%)	Lymphocytes (%)	Monocytes (%)	Eosinophils (%)	MCHC(%)
(Control)	$47.25\pm2.06_b$	$16.00\pm0.81_a$	7.05 ± 2.75^a	$55.75\pm6.34_a$	$42.25\pm7.27_a$	$2.50\pm0.57_a$	0.00 ± 0.00	33.86
(A)	$54.00\pm5.47_a$	$18.25\pm1.70_a$	6.12 ± 2.42^a	$59.50\pm5.25_{abc}$	$39.75\pm4.99_{ab}$	0.00 ± 0.00	$1.75\pm0.50_a$	33.80
(B)	$53.75\pm2.87_b$	18.25 ± 0.95^b	5.70 ± 2.15^a	$54.25\pm11.70_{ab}$	$44.75\pm10.53_a$	$3.50\pm1.29_b$	$0.oo\pm0.00$	33.95
(C)	$50.75\pm1.50_a$	17.25 ± 0.50^{ab}	5.12 ± 1.79^a	$49.75\pm13.22_{ab}$	$49.25\pm12.25_{ab}$	$3.50\pm1.00_{ab}$	0.00 ± 0.00	33.99

Control: Basal diet only; A: Basal diet + fungus only; B: Basal diet + fungus and bacterium; C: basal + bacterium only 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 ($p<0.05$)

Table 4: Effect of administration of *Fusarium nivale and Bacillus pumilus* on the haematological parameters of albino rats

Treatment	PCV (%)	Hb g L^{-1}	WBC $X10^9$/L	Neutrophils (%)	Lymphocytes (%)	Monocytes (%)	Eosinophils (%)	MCHC(%)
(Control)	$47.25\pm2.06_b$	$16.00\pm0.81_a$	$7.05\pm2.75_a$	$55.75\pm6.34_a$	$42.25\pm7.27_a$	$2.50\pm0.57_a$	0.00 ± 0.00	33.86
(A)	$52.50\pm5.19_a$	$17.75\pm1.89_a$	$8.25\pm4.86_a$	$44.75\pm6.99_a$	$54.00\pm5.59_c$	$2.00\pm0.00_a$	$1.75\pm0.95_{ab}$	33.81
(B)	$54.00\pm4.24_b$	$18.25\pm1.25_b$	$5.25\pm1.33_a$	$43.50\pm13.77_{ab}$	$54.75\pm13.02_a$	$3.00\pm1.41_{ab}$	0.00 ± 0.00	33.80
(C)	$50.00\pm2.44_a$	$17.00\pm0.81_a$	$6.87\pm1.65_{ab}$	$52.00\pm13.11_{ab}$	$46.00\pm15.01_{ab}$	$3.00\pm1.41_b$	$1.75\pm0.50_a$	34.00

Control: Basal diet only; A: Basal diet + fungus only; B: Basal diet + fungus and bacterium; C: basal + bacterium only 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 ($p<0.05$)

Treatment: Rats were randomly assigned to 4 test groups of 16 animals per group. One group (A) received oral administration of 0.3 mL of the fungus (10^7-10^8, respectively). The second group (B) were infected with 0.3 mL of the fungus and simultaneously treated with 0.3 mL of the bacterium. While the third group (C) rats were dosed (0.3 mL) with bacterium alone (broth cultures, 10^5-10^7cfu). The last group of animals were kept on basal diet alone and served as controls. The treatments were repeated again the second day. A post ingestion period of 14 days was observed after the administration. Throughout the study, all rats were housed in individual cages and had free access to water and food.

Sampling procedure: The rats were later sacrificed by cervical dislocation. Blood samples were collected into EDTA bottles. The haematological tests namely Packed Cell Volume (PCV), Haemoglobin (Hb) counts and White Blood Cell (WBC) counts were conducted according to the conventional method[10].

Statistical analysis: The data was expressed as Mean±standard error (SE). All statistical analysis was done by one way analysis of variance (ANOVA), SPSS 10.0.

RESULTS

Table 1-7 show the results of the haematological studies of albino rats subjected to microbial antagonism. The Packed Cell Volume (PCV) and Haemoglobin (Hb) were significantly ($p<0.05$) influenced by the bacteria treatment. The PCV of rats singly dosed were not significantly ($p>0.05$) different from those fed with control diet (Table 1-5). Among the treatments, rats dosed with *Aspergillus niger* and *Bacillus subtilis* had the lowest PCV and Hb. The White Blood Cell (WBC) counts of rats administered treatment B were significantly ($p<0.05$) lower than that of the control. The neutrophils, eosinophils, lymphocytes and monocytes of rats maintained on the treated diets were significantly ($p<0.05$) different from the

Table 5: Effect of administration of *Syncephalastrum racemosum* and *E. coli* on the haematological parameters of albino rats

Treatment	PCV (%)	Hb g L^{-1}	WBC $X10^9$/L	Neutrophils (%)	Lymphocytes (%)	Monocytes (%)	Eosinophils (%)	MCHC(%)
(Control)	$47.25\pm2.06_a$	$16.00\pm0.81_a$	$7.05\pm2.75_a$	$55.75\pm6.34_a$	$42.25\pm7.27_a$	$2.50\pm0.57_a$	0.00 ± 0.00	33.86
(A)	$49.50\pm3.00_a$	$17.00\pm1.15_a$	$7.60\pm2.10_a$	$62.25\pm6.50_{bc}$	$37.25\pm4.99_{ab}$	$3.25\pm0.50_a$	0.00 ± 0.00	34.34
(B)	$54.25\pm2.06_b$	$18.25\pm0.95_b$	$5.62\pm1.45_a$	$52.25\pm6.60_{ab}$	$46.25\pm4.78_a$	0.00 ± 0.00	$2.00\pm0.81_a$	33.64
(C)	$58.25\pm4.78_{ab}$	$19.00\pm0.81_c$	$7.75\pm1.74_{ab}$	$60.25\pm5.197_b$	$43.75\pm5.18_{ab}$	$2.50\pm0.57_{ab}$	0.00 ± 0.00	32.62

Control: Basal diet only; A: Basal diet + fungus only; B: Basal diet + fungus and bacterium; C: basal + bacterium only 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 ($p<0.05$)

Table 6: Effect of administration of *Rhizoctonia* sp. and *S. aureus* on the haematological parameters of albino rats

Treatment	PCV (%)	Hb g L^{-1}	WBC $X10^9$/L	Neutrophils (%)	Lymphocytes (%)	Monocytes (%)	Eosinophils (%)	MCHC(%)
(Control)	$47.25\pm2.06_b$	$16.00\pm0.81_a$	$7.05\pm2.75_a$	$55.75\pm6.34_a$	$42.25\pm7.27_a$	$2.50\pm0.57_a$	0.00 ± 0.00	33.86
(A)	$51.50\pm1.73_a$	$17.50\pm0.57_a$	$4.75\pm1.19_a$	$48.50\pm13.89_{ab}$	$50.25\pm12.94_{bc}$	$4.75\pm0.50_b$	0.00 ± 0.00	33.98
(B)	$51.50\pm3.87_{ab}$	$17.50\pm1.29_{ab}$	$8.50\pm4.12_a$	$61.00\pm\ldots$	$52.50\pm16.21_a$	0.00 ± 0.00	$3.75\pm2.06_b$	33.98
(C)	$52.50\pm2.87_b$	$17.75\pm0.95_{abc}$	$5.30\pm0.57_{ab}$	$41.50\pm4.79_a$	$59.75\pm6.13_b$	$4.75\pm0.50_b$	$2.00\pm0.81_a$	33.81

Control: Basal diet only; A: Basal diet + fungus only; B: Basal diet + fungus and bacterium; C: basal + bacterium only 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 ($p<0.05$)

Table 7: Effect of administration of *Penicillium chrysogenum* and *Bacillus sphaericus*on the haematological parameters of albino rats

Treatment	PCV (%)	WBC $X10^9$/L	Hb g L^{-1}	Neutrophils (%)	Lymphocytes (%)	Monocytes (%)	Eosinophils (%)	MCHC(%)
(Control)	$47.25+2.06_b$	$7.05+2.75_a$	$16.00+0.81_a$	$55.75+6.34_a$	$42.25+7.27_a$	$2.50+0.57_a$	$0.00+0.00$	33.86
(A)	$54.00+4.89_a$	$4.42+0.43_a$	$18.25+1.50_a$	$51.00+13.97_{abc}$	$47.25+13.37_{abc}$	$3.00+0.81_b$	$2.00+0.81_{ab}$	33.80
(B)	$53.75+2.87_b$	$5.07+2.01_a$	$18.25+0.95_b$	$55.00+13.68_{ab}$	$44.50+14.24_a$	$0.00+0.00$	$2.50+1.00_{ab}$	33.95
(C)	$55.50+3.31a_b$	$5.95+1.97_{ab}$	$18.75+0.95_{bc}$	$60.75+9.42_b$	$37.25+7.36_a$	$3.75+1.50_{ab}$	$1.75+0.95_a$	33.78

Control: Basal diet only; A: Basal diet + fungus only; B: Basal diet + fungus and bacterium; C: basal + bacterium only 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 ($p<0.05$)

control diet. No significant difference was observed in the MCHC values among the treatments.

DISCUSSION

The PCV, Hb, WBC, neutrophils, eosinophils, lymphocytes and monocytes compared favourably with the standard[11-13]. Aning *et al.*[10] and Oboh and Akindahunsi[14] 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 aberration caused in rats dosed with fungus alone could be due to the possible secretion of mycotoxins. Fungi are known to produce toxins. Aflatoxin B1 has been implicated in liver damage[15]. It appears that anything affecting protein utilization automatically reduces haematopoiesis. Reduced blood cell counts were recorded on agranulocytes (lymphocytes, monocytes) and granulocytes including eosinophils and neutrophils from group of rats administered same treatment were significantly ($p<0.05$) lower than that of the control in most groups. The low WBC count is an added advantage since increased numbers are associated with infections and leukamias. Decrease in WBC count normally reflects a decline in the production of defensive mechanism to combat infections. Such decrease will naturally make the rats more susceptible to various physiological stresses resulting in disease, poor growth and greater mortality[16]. Neither infection nor mortality was observed from the study, it appears that irrespective of the increased WBC counts (Table 1, 2 and 5) administered treatment C, the disruption in the metabolism of the various cell types involved in the defence mechanism would predispose the animals to infection if there was any attack. On the other hand, leucocytosis may occur as an indication of immunological response against foreign bodies. However, the WBC of the fungus infested rats (treatment A) was significantly lower than that of control. This low WBC count is another advantage to the use of micro fungi, since some of these fungi are capable of secreting antimicrobial substances that would restrict the growth of any contaminated organism. The lack of significant difference in MCHC among treatments using microbial antagonists' points to the fact that all blood samples collected for analysis had identical haemoglobin content. This result also agrees with Aletor[17] and Agbede[18]. The improvement in blood composition that followed feeding of the animals with treatment B indicated an immunological security for the group of animals given such treatment.

REFERENCES

1. Sorrels, K.M. and M.L. Speck, 1970. Inhibition of *Salmonella gallinarum* by culture filtrates of *Leuconostoc citrovorum*. J. Dairy Sci., 59: 338-343.

2. Gilliland, S.E. and M.L. Speck, 1977. Antagonistic action of *Lactobacillus acidophilus* towards intestinal food borne pathogens in associative cultures. J. Food Protec., 40: 820-823.
3. Klaenhammer, T.R., 1988. Bacteriocin of lactic acid bacteria. Biochimic, 70: 337-349.
4. De Vuyst, L. and E.L. Vandamme, 1994. Antimicrobial potential of Lactic Acid Bacteria Ed. London: Blackie Academy and Professional, pp: 91-142.
5. Shenin, I.D., L.F. Kruglikova, G.N. Kal'ko and Novikova, II, 1995. The characteristics of A alirin B-1-the basic component of a fungicidal preparation produced by the *Bacillus subtilis* 10-VIZR strain. Antibiotikii Khimioterapiya, 151: 159-165.
6. Agarry, O.O., F.A. Akinyosoye and F.C. Adetuyi, 2005. Antagonistic properties of microorganisms associated with cassava *(Mahinot esculenta*, Crantz) products. African J. Biotechnol., 4: 627-632.
7. Dicken, F. and H.E. Jones, 1981. Carcinogenic activity a series of reactive lactones and related substances. British J. Cancer, 15: 85.
8. Olowoyo, O.O., F.A. Akinyosoye and F.C. Adetuyi, 2004. Microorganisms associated with some cassava *(Manihot esculenta*, Crantz) products. J. Res. Rev. Sci., 2: 10-14.
9. Fujiwara, S., Y. Seto, A. Kimura and H. Hastuba, 2001. Establishment of orally-administered *Lactobacillus gasseri* SBT2055SR in the gastrointestinal tract of humans and its influence on intestinal microflora and metabolism. J. Applied Microbiol., 90: 343-352.
10. Aning, K.G., A.G. Ologun, A. Onifade, J.A. Alokan, A.I. Adekola and V.A. Aletor, 1998. Effect of replacing dried brewer's grains with sorghum rootlets on growth, nutrient utilization and some blood constituents in the rat. Anim. Feed Sci. Tech., 71: 185-190.
11. Mitruka, B.M. and H.M. Rawnsley, 1977. Clinical biochemical and haematological reference values in normal experimental animals. Masson, New York.
12. Baker, H.J., J.R. Lindsey and S.H. Wersbroth, The laboratory rat In: Biology and Diseases (Eds.) Academic Press New York.
13. Weihe, W.H., The laboratory rat In: UFAW Handbook on the Care Management of Laboratory Animals 6th Edn. (T.pool, Eds.), Longman Scientific and Technical, Harlow, UK. Williams and Wilkins Co. Baltimore, MD.
14. Oboh, G. and A.A. Akindahunsi, 2004. Nutritional and toxicological evaluation of *Saccharomyces cerevisiae* fermented cassava flour. J. Food Compos. Ana. (Short Commun), pp: 1-9.
15. Oboh, G., A.A. Akindahunsi and A.A. Oshodi, 2000. Aflatoxin and moisture content of micro-fungi fermented cassava products (flour and gari). Applied Tropical Agric., 5: 154-157.
16. Agrawal, N.K. and C.I. Mahayan, 1980. Haematological changes due to Vitamin C deficiency *Chana punctatus* block. J. Nutr., 110: 2172-2182.
17. 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. Intl. J. Food Sci. Nutr., 44: 289-292.
18. Agbede, J.O., 2004. Evaluation of growth characteristics and haematological variables of broiler-chickens fed diets containing groundnut cake replaced with either cooked jack bean meal or roasted devil bean meal. Biosci. Res. Commun., 16: 17-23.