INHIBITING EFFECTS AND BACITRIOCIN FACTORS PRODUCED BY LACTOBACILLI AGAINST SOME ENTERIC PATHOGENIC BACTERIA

[1]OBIAZI H.A.K., [1]UGWUOKE H.C., [1]EBADAN M.I., [1]OSULA O.T

[1]DEPARTMENT OF MEDICAL LABORATORY SCIENCE;
FACULTY OF BASIC MEDICAL SCIENCES
AMBROSE ALLI UNIVERSITY EKPOMA, EDO STATE, NIGERIA
Corresponding author email: hilarycu2000@gmail.com.

ABSTRACT

Lactobacilli are known probiotics and have been reported to have antimicrobial properties. They are present in fermented foods, fruits, gastrointestinal tract of humans and animals. This study was designed to access a certain fermented food in Nigeria, Fura de nunu, Ogi, Ugbore, palm wine, yoghurt and salad as a potential source of lactobacilli and investigate the action of its bacterial cell free supernatant (CFS) on certain organisms implicated in gastro enteric infection. These food products were analysed by cultural methods for the presence of lactobacilli and isolates were subjected to series of morphological biochemical tests. The analysis showed that all the food sources tested yielded lactobacilli except Ogi and salad which did not yield lactobacilli. Cell free supernatant (CFS) from de Man Rogosa Sharpe (MRS) broth cultures of the lactobacilli strains were used to challenge enteric bacteria by the agar well diffusion method and the mean diameter of zone of inhibiton determined such as *klebsiella species, shigella species, salmonella species, staphylococcus* and *E. coli* having mean and standard deviation (X±SD) of 3.40±1.14, 4.6±0.89, 4.0±1.0, 1.8±0.84 and 5.2±1.79 in millimeters respectively. E.coli had the highest (5.20mm) while the least was observed with *S.aureus* (1.8mm). the antbacterial susceptibility testing using lactobacilli was compared with those of standard antibiotics and the correlation was found to be positively associated (r= 0.92) for *shigella spp.* and other organism, except *S. aureus* which showed weak negative correlation (r= -0.281).

Key words: Lactobacilli, Probiotics, Antimicrobial, gastrointestinal, Infection

{**Citation:** Obiazi, H. A. K.; Ugwuoke, H.C.; Ebadan M.I.; Osula O.T. Inhibiting effects and bacitriocin factors produced by lactobacilli against some enteric pathogenic bacteria. American Journal of Research Communication, 2016, 4(9): 90-102} www.usa-journals.com, ISSN: 2325-4076.

INTRODUCTION

The art of the use of bacteria to kill or inhibit the growth of another type of organism such as bacteria is referred to as probiotic activities (Wiki, 2014). The lactic acid bacteria (LAB) comprise a class of Gram-positive, acid-tolerant, generally non-sporulating, non-respiratory rod or cocci that are associated by their common metabolic and physiological characteristic (Sonomotu and Yokota, 2011). These bacteria usually found in decomposing plants and lactic products, produce lactic acid as major metabolic end products of carbohydrate fermentation (Sonomotu and Yokota, 2011).

Proteinaceous bacteriocin are produced by several LAB strains and provide an additional hurdle for spoilage and pathogenic micro organisms. The genera that comprise the LAB are at its core Lactobacillus, Leuconostoc, Pedococcus, Lactococcus and Streptococcus as well as the more peripheral Aerococcus, Carnobacterium, Enterococcus, Oenococcus, Sporolactobacillus, Tetragenococcus, Vagococcus and Weisella, these belong to the order Lactobacillales (Sonomotu and Yokota, 2011).

Bacteriocins are proteinaceous toxins poduced by bacteria to inhibit the growth of similar or closely related bacterial strain(s). They are typically considered to be narrow spectrum antibiotics, though this has been debated (Farkas-Himsley, 1980). They are pheumenologically analogus to yeast and paramecium killing factors and structurally, functionally and ecologically diverse. Bacteriocins were first discovered by A. Gratia in 1925 (Gratia, 2000). He was involved in the process of searching for ways to kill bacteria which also resulted in the development of antibiotics and its discovery of bacteriophage all within a span of a few years. He also called his first discovery a collicin because it killed *E. coli* (Ljungh and Wadstrom, 2009).

MATERIALS AND METHODS

This study was carried out on isolates from Irrua Specialist Teaching Hospital (ISTH) and other Hospitals in Esan West Local government Area, a semi-urban town located in Esan local government area of Edo State. The major occupation of the people in this area are farming, trading and civil service.

This study involved total samples of 25 which included five (5) each of *Staphylococcus aureus, E.coli, Klebsiella spp, Salmonella spp* and *Shigella spp.*

The sample was suspended in 20mls of sterile Man Pogosa Sharpe (MRS) broth incubated microaerophillically for 24hours at 37^0C. A loopful of this was then inoculated on the plate of MRS agar. The plates were then incubated microaerophillically at 37^0C for 48hours. Afterwards, the isolates were selected and characterised on the basis of morphological, cultural and biochemical characteristics and were identified with the help of Bergey's Manual of Systematic Bacteriology.

The antibacterial effects of lactobacilli on pathogenic enteric organism were determined by the agar well diffusion method.

Nutrient agar plate was flooded with 0.5% MacFarland's standard for each of the Test bacteria and the excess was drained off. When the surface of the plate was dried, well (holes) were bored using gel puncher. The wells were layered with two drops of sterile molten nutrient agar and allowed to set. A 48hours broth culture of lactobacili was centrifuged at 1000rpm for 30minutes to obtain cell free supernatant of lactobacilli. Two drops of the cell free supernatant of lactobacilli culture was transferred into wells. It was then incubated at 37^0C for 24hours. Control was set along this experiment. The control comprised of uninnoculated MRS broth treated the same way as the lactobacilli culture. After the incubation period, zones of inhibition were measured in millimeter (Adeniyi *et al.*, 2006) and was compared with zones of inhibition of some standard antibiotics like Ciprofloxacin, Gentamycin, Streptomycin and so on.

RESULTS

The lactobacilli used were all isolated from fermentable food sources like, Ugbore (locust beans), Ogi, Fura de Nunu, Akamu (pap), Palm wine and salad, all samples yielded growth of Lactobacilli except Ogi and Salad.

Table 1. shows the diameter, mean and standard deviation of sensitivity pattern of some tested enteric bacteria with lactobacilli

S/N	***Klebsiella* spp (mm)**	***Shigella* spp (mm)**	***Salmonella* spp (mm)**	***S. aureus* (mm)**	***E.coli* (mm)**
1	3	5	4	2	6
2	4	4	3	1	5
3	3	4	3	2	4
4	5	6	5	3	5
5	2	4	5	1	6
X±SD	**3.40±1.14**	**4.60±0.89**	**4.00±1.00**	**1.80±0.84**	**5.20±1.79**

Keys:

X=mean, SD= Standard deviation

Table 2 Diameter of Sensitivity Pattern of Tested Enteric Bacteria With Some Standard Antibiotics

Antibiotics	*Klebsiella* (mm)	*Shigella* (mm)	*Salmonella* (mm)	*S.aureus* (mm)	*E.coli* (mm)
CIP	1	2	4	1	3
NIT	1	1	1	1	1
GEN	8	7	9	2	7
AMX	1	1	6	3	1
STR	6	2	8	3	7
RFN	1	6	5	2	4
ERY	3	2	5	1	1
CHL	3	1	1	1	1
AMP	3	1	1	1	1
LEV	3	5	4	1	5

Table 3 Correlation Table Showing the Relation between the Mean Diameter of the Activities of Lactobacillus and Standard Antibiotic on the Tested Organisms

Isolates	Lactobacilli X±SD	Std antibiotic X±SD	r-value	p-value	Remarks
E.coli	5.2±1.79	5.2±0.84	0.134	0.830	NS
S.aureus	1.8±0.84	1.8±0.84	0.429	0.472	NS
Salmonella	4.0±1.00	6.4±2.07	-0.241	0.696	NS
Shigella	4.6±0.89	3.8±2.49	0.92	0.634	NS
Klebisella	3.4±1.14	4.8±3.56	-0.283	0.645	NS

Keys: X=mean, SD= Standard deviation, r = co-efficient of correlation, NS=Not Significant

Table 4 T- Student Table Showing the Relation between the Mean Diameter of the Activities of Lactobacillus And Standard Antibiotic on the Tested Organisms

Isolates	Lactobacilli X±SD	Std antibiotic X±SD	T-Cal	p-value	Remarks
E.coli	5.2±1.79	5.2±0.84	0.000	1.00	NS
S.aureus	1.8±0.84	1.8±0.84	0.000	1.00	NS
Salmonella	4.0±1.00	6.4±2.07	2.59	0.061	NS
Shigella	4.6±0.89	3.8±2.49	-0.718	0.512	NS
Klebisella	3.4±1.14	4.8±3.56	0.878	0.429	NS

Keys: X=mean, SD= Standard deviation, T-Cal=T-student calculated, NS=Not Significant

Table 5 Isolation of Lactobacilli from Different Food Samples

FOOD SOURCES	*LACTOBACILLI*
Ogi	Absent
Ugbore	Present
Fura de Nunu	Present
Palm wine	Present
Salad	Absent
Yoghurt	Present
Akamu	Present

Table 6 Isolation of Pathogenic Organisms from Clinical Samples

Sample	*Salmonella spp*	*E. coli spp*	*Shigella spp*	*Staph. aureus*	*Klebsiella spp*
Blood	1	0	0	0	**0**
Stool	4	2	5	5	**5**
Urine	0	3	0	0	**0**
TOTAL	**5**	**5**	**5**	**5**	**5**

Table 7 The Prevalence of Lactobacilli from Different Food Samples

FOOD PRODUCTS	NUMBER	NUMBER POSITIVE	PERCENTAGE (%)
Akamu	10	10	**100**
Yoghurt	10	8	**80**
Ogi	10	0	**0**
Ugbore	10	10	**100**
Fura de Nunu	10	10	**100**
Palm wine	10	10	**100**
Salad	10	0	**0**

X2 value = 19.667

p-value = 0.0032

df = 6

level of significance = 0.05

t-tabulated = 12.592

DISCUSSION

Lactobacilli are frequently isolated from fermented foods, dairy/poultry products and GIT of animals and humans. They may beneficially affect the host upon ingestion by a variety of proven mechanisms (Fayol – Messaoudi *et al.,* 2005). From the health point of view, ingestion of live cells of *Lactobacilli* in adequate amount is believed to confer several beneficial physiological effects on the host (Tannock, 2004). The criteria for the in vitro selection of *Lactobacilli* in food and pharmaceutical preparations include antibiotics tolerance as well as the production of lactic acid that inhibit growth of microorganisms including UTI causing organisms (Cotanzaro and Green, 1997). This present study examined the antagonistic effect of *Lactobacilli* and comparative activity with standard antibiotic against some enteric bacteria.

In this study, the morphological and biochemical analysis confirmed that all the isolates from the fermentable foods except for salad and Ogi did yield members of the genus *Lactobacilli*. This could be so because all the foods were derived from fermentable sources and *Lactobacilli* can be found in fermented food products (Adeniyi *et al.,* 2006).

Lactobacilli isolated showed weak inhibitory effect (mean diameter < 10mm) on all the tested organism of *Klebsiella* species, *Shigella* species *Salmonella* species, *Staphylococcus aureus* and *Escherichia coli* and this founds were in agreement with the independent research conducted by Ozlem and Feryal, 2005, *Lactobacilli* species also had weak inhibitory effect (mean diameter < 2mm) on *S. aureus.* Selvamohan *et al*. (2010) also reported that, *Lactobacilli* strains had inhibitory effect on the above organisms. A study by Ashraf *et al*. (2009) also revealed that *Lactobacilli* strains have antagonistic effect against *E. coli* and *S. aureus* with zone of inhibition less than 10mm. The antimicrobial action is due to the potential of *Lactobacilli* to produce lactic acid and bacteriocins. It was also reported that these bacteria produce peptides that have inhibitory properties (Strus *et al*., 2001).

In this study, it was also found that the different microorganisms tested had varied inhibitory spectrum, for instance, *E. coli* was the most sensitive (mean diameter of 5.20mm) while *S. aureus* was the least sensitive (mean diameter of < 2mm).The reason for this variation in the degree of inhibition could be that some of them could thrive better in the acidified environment produced by *Lactobacilli*. For instance, *S. aureus* was the least sensitive to *Lactobacilli*; this could probably be because *S. aureus* thrive better in a salty environment (Mobley and Chippendale, 1991) and other organisms that utilized urea could have neutralized the potency of the lactic acid in *Lactobacilli*, which resulted into the very weakest inhibition observed in this study.

Gentamycin is active against a wide range of human bacterial infections, mostly Gram-negative bacteria, but is was found to be resistant to most of the tested organism and this could be due to the fact as it has be reported that most enteric bacteria had acquired resistant genes which must have be acquired from other organisms in the enteric region as asserted by Moulds *et al.*,(2010) and Nitrofuranton which is active against most urinary tract pathogens were found to be least active against the same type of enteric organism which they are active to when found in the urinary tract or other sites expect enteric sources. Relationship between mean diameter of zone of inhibition of the tested organisms with activities of *Lactobacilli* and those of the standard antibiotics, the activities of *Lactobacilli* and standard antibiotics on *Escherichia coli* have a weak positive insignificant correlation of 0.134 (p>0.05), on *Staphylococcus aureus* showed a strong positive correlation value (r=0.429, p>0.05) of insignificant zone of inhibition, on *Klebsiella* species, showed a weak negative correlation (-0.283), on *Shigella* species, a great positive correlation value (r=0.92, p>0.05) was showed while *Salmonella* species showed a weak negative correlation (-0.241) respectively when the mean diameter of zone of inhibition on the tested organisms with activities of *Lactobacilli* and those of the standard antibiotics were compared correlationally.

The strong correlation showed by both the mean zone inhibition by *Lactobacilli* and standard deviation on *Shigella* species (r=0.92) may not be unconnected to the fact that *shigella* species produces toxin which are inactivated with either acidic or alkaline medium (Mounier, 1992), and all the positive correlation between the activities of *Lactobacilli* and the standard antibiotic on the tested organisms suggest that there are positive relationship between the mode of action or mechanism of action of *Lactobacilli* and the standard antibiotic and the insignificant differences between them showed that there are no great differences in the inhibition process of the tested organisms by both *Lactobacilli* and the standard antibiotic, while those with negative correlation showed that there were association but it was not significant, and all this findings were in agreement with the work of some researchers like Piard and Desmazeaud,1992, Adeniyi *et al.*, (2006) and Khay *et al.*, (2011) who reported same in their independent findings, although they were contrary to the findings of Fayol-Messaoudi *et al.*, (2005).

This resistance could have resulted from factors that affect in vitro susceptibility testing such as size of inoculum, concentration of antibiotic agent, period and temperature of incubation, etc. (Ochei and Kolhatker, 2008).

CONCLUSION

Evidence from the results obtained has shown that fermentable food sources contain *Lactobacilli* expect salad and these *Lactobacilli* have antagonistic effect against certain some enteric pathogenic bacteria owing to their ability to produce some inhibitory substances like lactic acids and bacteriocins. However this study only determined the inhibitory effect of *Lactobacilli* against these organisms and compared the mean zone of inhibition with some standard antibitioics further studies need to be done using more sensitive methods (molecular methods) such as Polymerase chain reaction that will identify these *Lactobacilli* to their species level. *Lactobacilli* was found to be most active against *Escherichia coli* with a mean diameter zone of (>5.20mm) and a weak positive insignificant correlation of 0.134 ($p>0.05$). While *Staphylococcus aureus* had the least mean zone of inhibition (<1.8mm). When compared with standard antibiotics it was found that there was a positive association between them statistically.

RECOMMENDATION

From this study, the benefit of *Lactobacilli* as a probiotic to human health cannot be overemphasized. It has benefits like anti-carcinogenic and antimicrobial properties, it has been found to be effective against diarrhea and several other ailments. It is therefore recommended that; Awareness should be created by health professionals to the general public on the benefits associated with the consumption of these organisms and also the foods that contain them. A thorough study should be done to compare the actual mechanism of action/mode of action of *Lactobacilli* to pathway; for a possible inclusion as a potent antibiotic after purifications. The pharmaceutical industry should explore its antibacterial activity in future manufacture of new antibiotics.

REFERENCES

Adeniyi, B.A., Ayeni, F.A. and Ogunbanwo, S.T. (2006): Antagonistic Activities of Lactic Acid Bacteria Isolated from Nigerian Fermented Diary food against Organisms Implicated in Urinary Tract Infection. *Biotech. 5*: 183- 188.

Ashraf, M., Arshad, M., Siddique, M. and Muhammed, G. (2009): Invitro screening of Lactobacilli spp for probiotics activities. *Pakis. Vet. J. 29(4):* 186-190.

Catanzaro, J. and Green, L. (1997): Microbial ecology and probiotics in human medicine (Part II).*Altern.Med. Rev. 2(4)*: 296-305.

Farkas-Himsley, H. (1980): "Bacteriocins--are they broadspectrum antibiotics?", *J. Antimicrob. Chemother*. **6** (4): 424–426.

Fayol-Messaoudi, D., Berger, C.N., Coconnier-Polter, M., Liévin-Le Moal, V. and Servin, A.L. (2005): Lactic Acid and Non-Lactic Acid-Dependent Activities of Probiotic Lactobacilli against *Salmonella enterica* Serovar Typhimurium. *Appl. Environ. Microbiol*. *71*: 6008-6013.

Gratia, J.P. (2000): "André Gratia: a forerunner in microbial and viral genetics, *Genetics* 156 (2): 471–476.

Ljungh, A and Wadstrom, T. (2009): Lactobacillus Molecular Biology: From Genomics to Probiotics. Caister Academic Press. ISBN 978-1-904455-41-7.

Moulds, R. and Jeyasingham, M. (2010). "Gentamicin: a great way to start". Australian Prescriber (33): 134–135.

Mounier, J. (1992). "Shigella flexneri Enters Human Colonic Caco-2 Epithelial Cells through the Basolateral Pole". Infection and Immunity 60 (1): 237–248.

Ochei, J. and Kolhatker, A. (2008): Diagnosis of Infection by Specific Anatomic Site. In: Medical Laboratory Science. Theory and Practice. 1st ed. Tata McGraw Hill Publishing Company Limited, New Delhi (Publisher). Pp. 636-638.

Sonomoto, K. and Yokota, A.(2011): *Lactic Acid Bacteria and Bifidobacteria: Current Progress in Advanced Research*. Caister Academic Press. ISBN 978-1-904455-82-0.

Tannock, G. (2005): Probiotics and Prebiotics: Scientific Aspects (1st ed.). Caister Academic Press. ISBN 978-1-904455-01-1.