American Research Journal of Food and Nutrition

Volume 3, Issue 1, 1-16 Pages
Research Article | Open Access

AMERICAN RESEARCH JOURNALS
An Academic Publishing House

Probiotic Foods and their Health Benefits

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ABSTRACT

Probiotics have been defined a number of times. Presently, the most common definition is that from the Food and Agriculture Organization (FAO) and the World Health Organization (WHO) (2012) which states that probiotics are live microorganisms that when administered in adequate amounts, confer a health benefit on the host. Probiotic bacteria are found in the human gut where they provide health benefits to the host. They do so by producing nutrients and cofactors, successfully competing with pathogens and stimulating host immune responses by producing specific polysaccharides. These bacteria can also alleviate the symptoms of disease-related disorders.

This study was carried out on the health benefits derived from the use of probiotics. Food such as the fermented African Nightshade and two different fermented juices were analyzed in the laboratory so as to determine the different types of probiotics that are found in them and also their interaction with pathogenic bacteria (*Escherichia coli and Staphylococcus aureus*). These pathogenic bacteria were cultured with the probiotic bacteria in order to determine their interaction. Microorganisms derived from the fermented vegetables were cultured in nutrient agar media. For the fruit juices, serial dilution was done, subjected to culture in an incubator for 24 hours and later identification of the bacteria by their morphological characteristics, biochemical tests, Gram staining technique and also by Bergey's Manual of determinative bacteriology.

The probiotics that were present in the fermented juices included *Pediococcus spp, Enterococcus spp, Bacillus spp, Bifidobacterium spp, Lactobacillus spp* and *Streptococcus spp*. While bacteria present in fermented African Nightshade (*Solanum villosum)* include *Leuconostoc spp* and *Lactobacillus spp*. These bacteria inhibit the growth of pathogenic bacteria by forming zones of inhibition. Therefore, the fermented juices and fermented African Nightshade (*Solanum villosum)* have probiotic bacteria that inhibit the growth of pathogenic bacteria.

INTRODUCTION

Background Information

The roots of the word probiotics come from the Greek word pro, meaning 'promoting' and biotic meaning 'life'. Therefore, probiotics as defined by the Food and Agriculture Organization of the United Nations (FAO) and World Health Organization (WHO) in 2012 comprise live microorganisms which when administered in adequate amounts, confer a health benefit on the host.

Regular consumption of food containing probiotic microorganisms is recommended to establish a positive balance in the population of useful or beneficial microbes in the intestinal flora. The composition of the micro biota at the beginning of human life can affect the health for several months. Although people often think of bacteria and other microorganisms as harmful 'germs', many microorganisms help our bodies function properly. For example, the bacteria that are usually present in our intestines help digest food, destroy disease-causing microorganisms and produce vitamins. Large numbers of microorganisms live on and in our bodies. In fact, research shows that microorganisms in the human body outnumber human cells by 10 to 1.

Recent scientific work on the properties and functionality of living microorganisms in food have suggested that probiotics play an important role in immunological, digestive and respiratory functions and that they could have a significant effect on the alleviation of infectious diseases in children and other high-risk groups. Although each area of our body is colonized by microorganisms, most of them lie in our gut. Gut micro biota is the terminology used to describe the huge amounts of microorganisms that colonize the entire digestive tract, (Evangelisti and Restani, 2011). Research has shown that probiotic bacteria can colonize and proliferate in the intestinal tract of humans and animals to prevent the growth of intestinal pathogens (Fuller, 1989).

Fermented milk and yogurt with probiotic products are the most popular in the market. However, there are other viable alternatives to products that do not contain milk, both to meet the needs of those people suffering from lactose intolerance or hypercholesterolemia and to meet the growing

demand for vegetarian products (Ranadheera *etal.*, 2010). For this reason, there is an increasing demand for vegetarian probiotic products, (Ranadheera *et al*, 2010).

Fermentation processes are believed to have been developed over the years by women in order to preserve food for times of scarcity, to impart desirable flavor to foods and to reduce toxicity, (Rolle and Satin, 2002). Food fermentation is regarded as one of the oldest ways of food processing and preservation. Throughout the ancient history, health promoting fermented foods has played a role in sustaining thriving civilizations. Fermentation enhances the unique flavor and nutritional quality of food. These foods are thought to be high in essential amino acids, sodium, fiber and calcium and contribute to a balanced lifestyle. Foods that are prepared by fermentation have long been shown to help retain shelf life and prevent food spoilage. Different bacterial species are present which contribute to the unique taste, flavors and texture present in fermented foods. Traditionally fermented food is the main source of probiotics and hence one of the major dietary supplements of modern world. Fermentation enhances the growth of beneficial microbes that beneficially affects the host – in this case, people- by improving their intestinal microbial balance thus contributing to the livelihoods of rural and urban dwellers alike.

This study therefore, aims to establish the potential use of fermented juices and fermented vegetables as probiotics.

MATERIALS AND METHODS

Sample Collection

Managu (African nightshade) was obtained from the market; the leaves were washed thoroughly and boiled and later the water used for boiling discarded. Fresh milk was then added to the vegetable and covered using a lid; this was then placed at room temperature. On the second day, fresh milk was then added to the vegetable and this continued until the fifth day when the vegetable reached the level of tartness. The vegetable was then taken to the laboratory for research.

For the fermented juices, cabbage, garlic, ginger, onion, beetroot and lemon were obtained from the market. Cabbage leaves, garlic, ginger and onion were used in the preparation of juice A. They were washed thoroughly, cut into small pieces and blended together. They were then put in a clean and sterilized plastic container, covered completely and left for fermentation to occur for four days. Beetroot and lemon were added to all contents to juice A making it juice B. They were then fermented. After the juices were fermented, they were taken in the laboratory for research.

LABORATORY WORK

Sterilization of Working Benches and Apparatus

Working benches were sterilized with hypochlorite and the glass apparatus and equipment were autoclaved at 121°c for 15 minutes.

Culturing of Bacteria

Two types of fermented juices; one with cream color (A) and another with pink color (B) were put in test tubes. 1ml of the juice sample was mixed with 9ml of sterile distilled water. This was then serial diluted from 10^{-1} to10^{-6}. This was also done for the other fermented juice sample.

1g of fermented vegetable African Nightshade (*Solanum villosum)* was put in a sterile mortar and crushed by the use of a pestle. This was put in a test tube and mixed with 10ml of sterile distilled water. 1ml was then taken and mixed with 9ml of sterile distilled water. This was then serial diluted from 10^{-1} to10^{-6}.

7gms of nutrient agar was put in a conical flask. It was then diluted with 250ml of distilled water, covered using a piece of cotton wool and aluminum foil and well mixed. This was then placed in an autoclave together with the petri dishes to be used and was sterilized at 121°c for 15 minutes.

Pour-plate method was used to culture bacteria from both the fermented juice samples and that of the vegetable sample so as to obtain mixed cultures of each sample. 1ml of each sample was measured and poured in the petri dishes. The Nutrient Agar (NA) media was then poured on the samples that were in the plates and allowed to solidify for some time. The nutrient agar plates were then incubated at 37° Celsius for 24h in an inverted position. The cultures obtained were mixed cultures of the bacterial colonies and they were identified by their morphological characteristics. The different types of bacterial colonies were then sub cultured so as to obtain pure colonies of the bacteria. Nutrient Agar (NA) was prepared and poured in the petridishes and allowed to solidify. An inoculating loop was sterilized using a hot flame before it was used to take part and streak on the surface of the NA. They were then incubated again in an inverted position for 24 hours. Bacteria from each sample were interacted with pathogenic bacteria of *E. coli* and *S. aureus.* Their interactions were observed by formation of inhibition zones that were measured from day one, three, five, seven and day ten.

Individual bacterial colonies were identified by their morphological characteristics, biochemical techniques, Gram staining technique and by using the taxonomic scheme of Bergey's Manual of Determinative Bacteriology, (Holt *et al*., 1994).

Gram Staining Technique

Gram staining was done as described by Coico, (2005). Single colonies obtained from the streaked plates were picked for the Gram staining. The picked colony was placed on a clean slide and heat fixed by passing the slide over a flame. A few drops of crystal violet were added to the heat fixed smear for 60seconds and rinsed with tap water. It was flooded with iodine solution for 60seconds and rinsed with tap water. The smear was decolorized with alcohol until there was no more

stain and rinsed with slow running tap water. Safranin was added to the smear for about 40seconds to counter stain and washed with tap water. The smear was then dried with soft paper; immersion oil was placed on the smear and observed under the microscope at x100. The shape and color of the cells were examined. Gram positive bacteria stains purple while the Gram-negative bacteria stains pink.

BIOCHEMICAL TESTS

i. Catalase Test

Catalase is an enzyme found in most bacteria. It catalyzes the breakdown of hydrogen peroxide with the release of free oxygen and water. The test is used to determine whether a bacterium can produce the catalase enzyme. A loop full of 24h old culture of each isolate was put on a clean slide. A drop of hydrogen peroxide was added to it. The production of bubbles shows the presence of catalase enzyme which is a positive result while a negative result is indicated by no bubble formation.

ii. Coagulase Test

Coagulase test is used to detect clumping factor. Clumping factor directly converts fibrinogen to fibrin causing agglutination. Bacterial colony was smeared on a clean slide and a drop of water added. If a coarse clumping of the bacteria is visible after a few seconds, then the reaction is positive. A negative result is shown by the absence of clumping or any reaction taking more than 10 seconds to develop.

iii. Indole Test

Indole test is performed to determine the ability of the organism to split tryptophan to form the compound indole. Indole is one of the metabolic degradation products of the amino acid tryptophan. Indole production is detected by Kovac's reagent which contains 4(p)-dimethylamino benzaldehyde, this reacts with indole to produce a red colored compound. An isolated colony of the test bacteria was emulsified in the tryptophan broth which was then incubated at 37°c for 24-28 hours. 0.5 drops of Kovac's reagent was added to the broth culture and the color change observed. The development of bright red color at the interface of the reagent is indicative of the presence of indole and is a positive test. A negative test is indicated by no color change and therefore no ring even after the addition of the reagent.

iv. Methyl Red Test (MR-Test)

Methyl red test is used to test whether the microbe performs mixed acids fermentation when supplied with glucose. Types and proportion of fermentation products produced by anaerobic fermentation of glucose is one of the key taxonomic characteristics which help to differentiate various genera of enteric bacteria. Test tubes containing MR broth with pure culture of the microorganisms under investigation were inoculated and incubated for 37°c for up to 4 days. 5 drops of methyl red indicator solution were added to each test tube. A positive test is shown when the culture medium turns red after addition of methyl red because of a pH at or below 4.4 from the fermentation of glucose. A negative result is indicated when the culture medium remains yellow, which occurs when less acid is produced (pH is higher) from the fermentation of glucose.

v. Citrate Utilization Test

Citrate utilization test is used to distinguish between members of the Enterobacteriaceae family based on their byproducts. This test is used to determine the ability of bacteria to utilize sodium citrate as its only carbon source and inorganic ($NH_4H_2PO_4$) as the sole fixed nitrogen source. When the bacteria metabolize citrate, the ammonium salts are broken down to ammonia, which increases alkalinity the shift in pH turns the bromthymol blue indicator in the medium from green to blue above pH of 7.6. A bacterial colony was inoculated on Simmons citrate agar lightly on the slant by touching the tip of the needle. This was incubated for 37°c for 4 days (although some bacteria were incubated up to 7 days due to their limited rate of growth on citrate medium). Color change was then observed from green to blue along the slant. A positive reaction is indicated by growth with color change from green to intense blue along the slant. In the negative reaction, no growth and no color change; the slant remains green.

RESULTS

Bacteria Isolated from Fermented Juice A

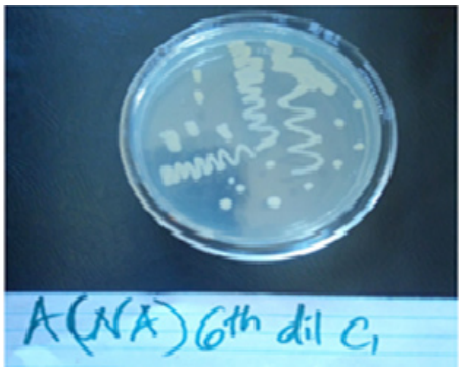


Fig 4.1. Pediococcus in fermented juice A

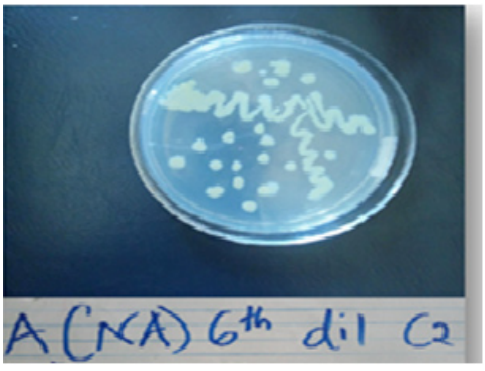


Fig 4.2. Enterococcus in fermented juice A

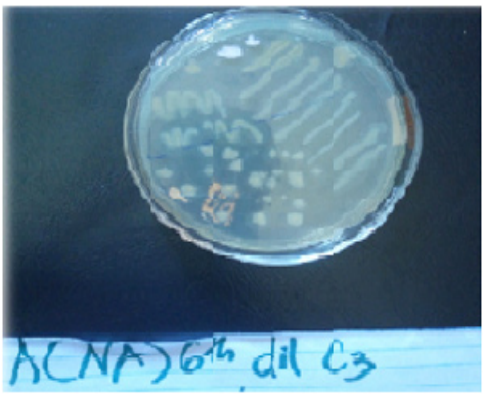


Fig 4.3. Bifidobacteria in fermented juice A

Figure 4.1 show *Pediococcus* bacteria which was identified from fermented juice A. The fermented juice was serial diluted up to the 6th dilution and then cultured in Nutrient Agar.

Figure 4.2 show *Enterococcus* bacteria that were identified from fermented juice A. The fermented juice was serial diluted up to the 6th dilution and then cultured in Nutrient Agar.

Figure 4.3 show *Bifidobacteria* which was isolated from fermented juice A.The fermented juice was serial diluted up to the 6th dilution and then cultured in Nutrient Agar.

Bacteria Isolated from Fermented Juice B

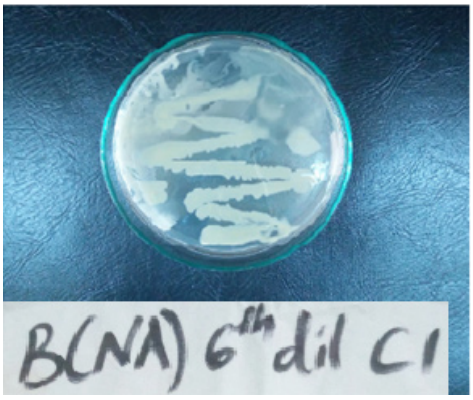


Fig 4.4. Bacillus in fermented juice B

Fig 4.5. Streptococcus in fermented juice B

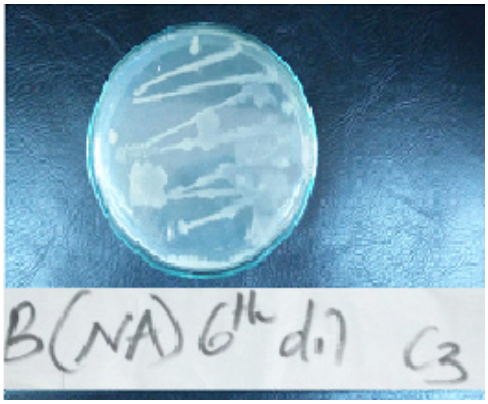


Fig 4.6. Lactobacillus in fermented juice B

Figure 4.4 show *Bacillus* bacteria that were obtained from fermented juice B. The fermented juice was serial diluted up to the 6th dilution and then cultured in Nutrient Agar.

Figure 4.5 show *Streptococcus* bacteria which was isolated from fermented juice B. The fermented juice was serial diluted up to the 6th dilution and then cultured in Nutrient Agar.

Figure 4.6 show *Lactobacillus* bacteria which was isolated from fermented juice B. The fermented juice was serial diluted up to the 6th dilution and then cultured in Nutrient Agar.

Bacteria Isolated from Fermented African Nightshade (*Solanum Villosum*), Managu is Swahili Local Name for the Vegetable

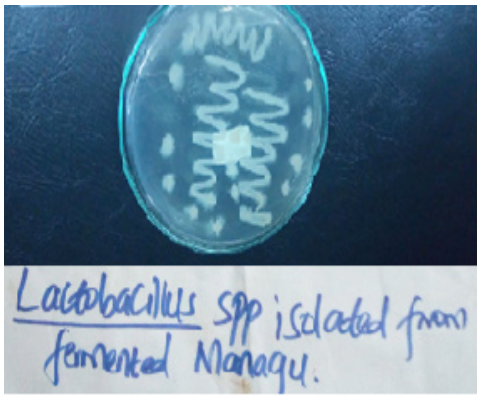


Fig 4.7. Lactobacillus in fermented S. villosum

Fig 4.8. Leuconostoc in fermented S. villosum

Figure 4.7 show *Lactobacillus* bacteria that was isolated from fermented *S. villosum*.

Figure 4.8 show *Leuconostoc* bacteria which was isolated from fermented *S. villosum*.

Biochemical Tests

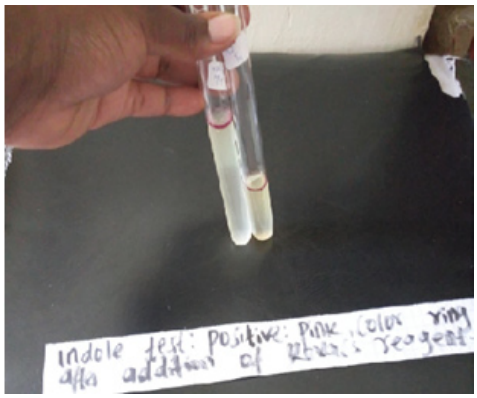


Fig 4.9. Indole positive results

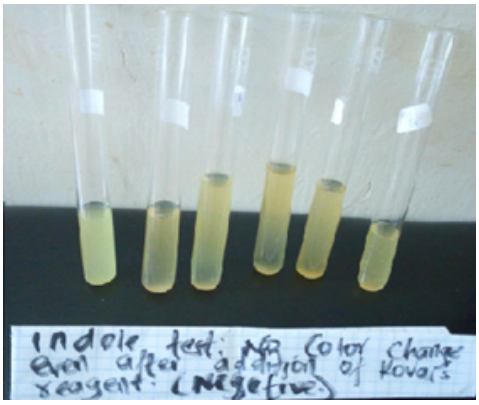


Fig 4.10. Indole negative results

Figure 4.9 show indole positive result which was in *Enterococcus* bacteria that was isolated from fermented juice A as shown in Figure 4.2 above.

Figure 4.10 show indole negative result. The negative results were in *Pediococcus* and *Bifidobacteria,* which were isolated from fermented juice A in Figure 4.1 and Figure 4.3 above; In addition to *Bacillus, Streptococcus* and *Lactobacillus* which were isolated from fermented juice B and are shown in Figure 4.4, Figure 4.5 and Figure 4.6 above respectively.

Indole negative result was also in *Lactobacillus* and *Leuconostoc* bacteria which were isolated from fermented *S. villosum*as shown in Figure 4.7 and Figure 4.8 above respectively.

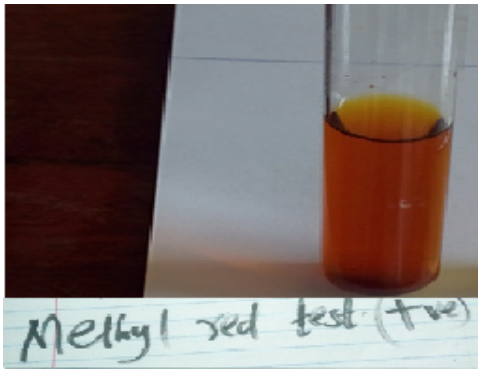


Fig 4.11. Methyl red test positive results

Fig 4.12 Methyl red test negative results

Figure 4.11 show a positive result in methyl red test. Methyl red positive result was in *Pediococcus* species. This species is shown in Figure 4.1 above

Figure 4.12 show methyl red negative results which were in *Enterococcus* and *Bifidobacteria* which was isolated from fermented juice A. These bacteria are present in Figure 4.2 and Figure 4.3; In addition to *Bacillus, Streptococcus* and *Lactobacillus* bacteria which were isolated from fermented juice B as shown in Figure 4.4, Figure 4.5 and Figure 4.6 above respectively; Also, *Lactobacillus* and *Leuconostoc* bacteria which were isolated from fermented *S. villosum* as shown in Figure 4.7 and Figure 4.8 above respectively.

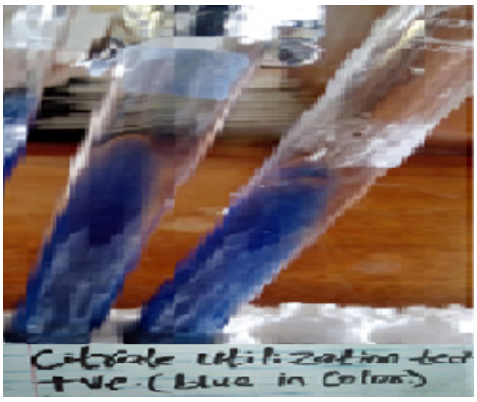


Fig 4.13. Positive results for citrate utilization test

Fig 4.14. Negative result for citrate utilization test

Figure 4.13 show citrate utilization positive test. This positive test was in *Bacillus* and *Streptococcus* bacteria that were isolated from fermented juice B as shown in Figure 4.4 and Figure 4.5 above respectively.

Figure 4.14 show citrate utilization negative results. The negative results were in *Pediococcus, Enterococcus,* and *Bifidobacteria,* which were isolated from fermented juice A as indicated by Figure 4.1, Figure 4.2 and Figure 4.3 above respectively; In addition, fermented juice B which had *Lactobacillus* bacteria as shown in Figure 4.6; *Lactobacillus* and *Leuconostoc* bacteria that were isolated from fermented *S. villosum* vegetables as shown in Figure 4.7 and Figure 4.8 above respectively also showed negative results.

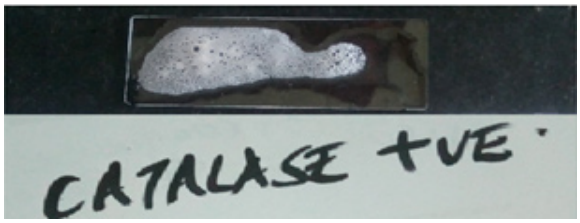


Fig 4.15. Catalase positive results

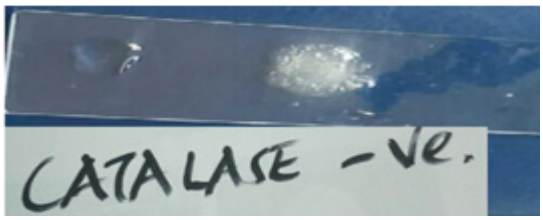


Fig 4.16. Catalase negative result.

Figure 4.15 show catalase positive result. *Pediococcus*bacteria that was isolated from fermented juice A and shown in Figure 4.1 above and *Bacillus* bacteria fromfermented juice B shown in Figure 4.4 above tested catalase positive.

Figure 4.16 showscatalase negative result. Catalase negative results were in *Enterococcus* and *Bifidobacteria* which was isolated from fermented juice A. These bacteria are present in Figure 4.2 and Figure 4.3 above;*In addition, Streptococcus* and *Lactobacillus* bacteria which were isolated from fermented juice B as shown in Figure 4.5 and Figure 4.6 above respectively also tested catalase negative. *Lactobacillus* and *Leuconostoc* bacteria which were isolated from fermented *S. villosum* vegetables as shown in Figure 4.7 and Figure 4.8 above respectively were also catalase negative.

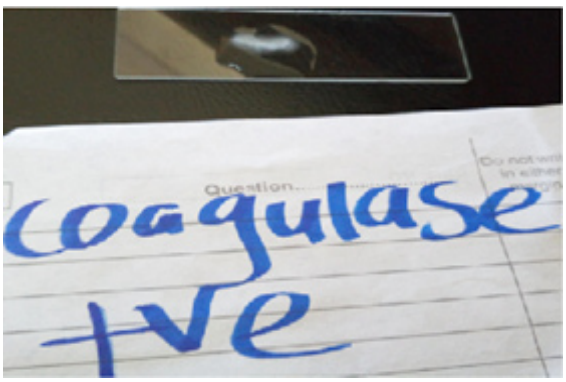


Fig 4.17. Coagulase positive result

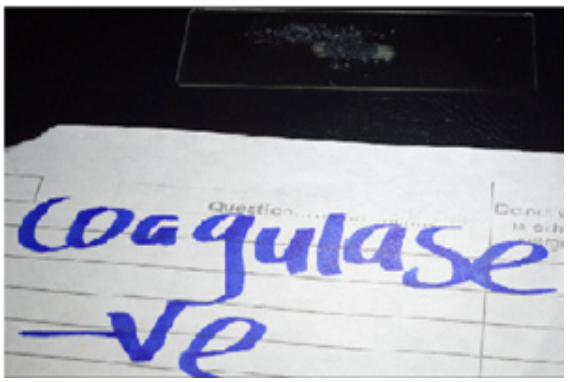


Fig 4.18. Coagulase negative result

Figure 4.17 show coagulase positive result. *Streptococcus* bacteria in Figure 4.5 from fermented juice B showed coagulase positive results.

Figure 4.18 show a negative result. Coagulase negative result were in a sample of fermented juice A, *Pediococcus, Enterococcus,* and *Bifidobacteria,* as indicated by Figure 4.1, Figure 4.2 and Figure 4.3 above respectively; In addition, *Bacillus* and *Lactobacillus* bacteria which were isolated from fermented juice B as shown in Figure 4.4, and Figure 4.6 above respectively; *Lactobacillus* and *Leuconostoc* bacteria which were isolated from fermented *S. villosum* vegetables as shown in Figure 4.7 and Figure 4.8 above respectively also showed negative results.

Interaction between Pathogenic Bacteria and Probiotics

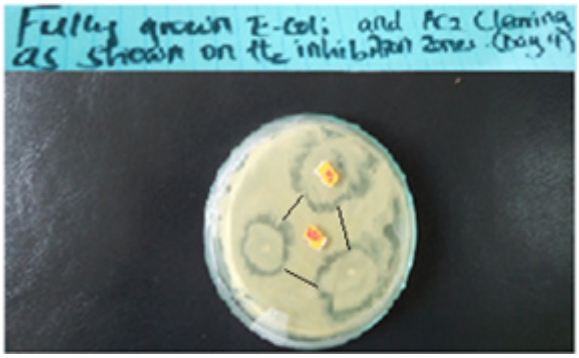


Fig 4.19. Enterococcus acting against E. coli

Fig 4.20. Enterococcus acting against S. aureus

Figure 4.19 show interaction between *Enterococcus* bacteria obtained from fermented juice A and as shown in Figure 4.2 put on the three spots on a petri dish with fully grown *E. coli.* The interaction is in such a way that *Enterococcus* bacteria inhibit the growth of *E. coli*. A zone of inhibition is measured

Fig4.20 show interaction between *Enterococcus* bacteria obtained from fermented juice A,as shown in Figure 4.2 interacting with *S. aureus*. An *Enterococcus* bacteriumis put on the three spots on a petri dish with fully grown *S. aureus.* The *Enterococcus* bacteria inhibit the growth of *S. aureus* by forming zones of inhibition.

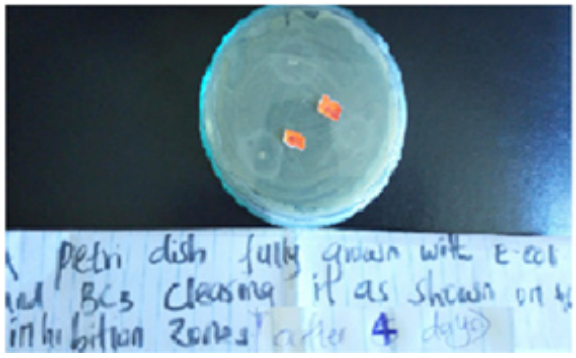


Fig 4.21. Lactobacillusacting against E. coli

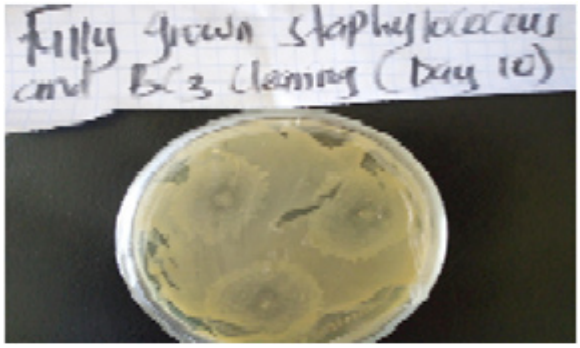


Fig 4.22. Lactobacillus acting against S. aureus

Fig4.21 show *Lactobacillus* bacteria which is in Figure 4.6 above from fermented juice B, interacting with *E. coli.Lactobacillus* bacteria inhibit the growth of *E. coli* as shown by the inhibition zones.

Fig 4.22 show interaction between *Lactobacillus* bacteria from fermented juice B and is in Figure 4.6 above which was acting against *S. aureus* by forming the zones of inhibition.

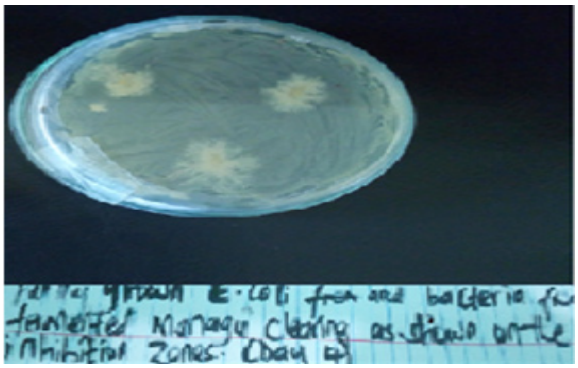


Fig 4.23. Leuconostocacting against E. coli

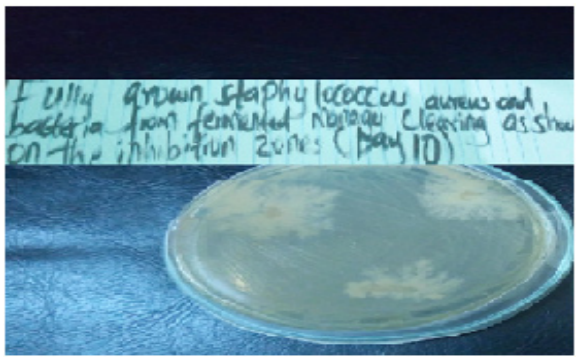


Fig 4.24. Leuconostocacting against S. aureus

Fig 4.23 show interaction between *Leuconostoc* bacteria from fermented *S. villosum* vegetables and which is in Figure 4.8 above interacting with *E. coli.Leuconostoc* bacteria prevents *E. coli* from growing by forming zones of inhibition

Fig4.24 show interaction between *Leuconostoc* bacteria from fermented *S. villosum* vegetables and which is in Figure 4.8 above interacting with *S. aureus. Leuconostoc* bacteria act against *S. aureus* by forming zones of inhibition.

Identification of Bacteria

Table 4.1. Table showing identification of bacteria

ISOLATES		COLOUR	ELEVATION	CATALASE	SHAPE	G STAIN	FORM	MARGIN	COAGULASE	INDOLE	MR	CITRATE	IDENTIFICATION
A	C1	Cream yellow	Raised	Negative	Cocci	G+Ve	Rhizoid	Filiform	Negative	Negative	Positive	Negaitive	*Pediococcus*
	C2	White	Flat	Positive	Cocci	G+Ve	Filamenticus	Filiform	Negative	Positive	Negative	Negative	*Enterococcus*
	C3	White	Convex	Negative	Bacillus	G+Ve	Circular	Entire	Negative	Negative	Negative	Negative	*Bifidobacteria*
B	C1	Cream white	Crateriform	Positive	Bacillus	G+Ve	Irregular	lobate	Negative	Negative	Negative	Positive	*Bacillus*
	C2	Cream white	Convex	Negative	Cocci	G+Ve	Irregular	Entire	Positive	Negative	Negative	Positive	*Streptococcus*
	C3	White	Flat	Negative	Bacillus	G+Ve	Filamenticus	Filiform	Negative	Negative	Negative	Negative	*Lactobacillus*
VEGES	C1	White	Flat	Negative	Cocci	G+Ve	Filamenticus	Filiform	Negative	Negative	Negative	Negative	*Leuconostoc*
	C2	White	Flat	Negative	Bacillus	G+Ve	Filamenticus	Filiform	Negative	Negative	Negative	Negative	*Lactobacillus*

These results show the probiotics that were found in fermented juices and fermented *S. villosum* vegetables and the characteristics that identifies each probiotic. The characteristics involve both the morphological characteristics and the biochemical tests that were carried out in their identification. The morphological characteristics include their color, elevation, shape, form and margin. The biochemical tests include the catalase test, Gram staining technique, coagulase test, indole test, methyl red test and the citrate utilization test.

Because there are probiotics found, I reject my null hypothesis that there are no probiotics in fermented vegetables and fermented juices.

Statistical Data Results

Zones of inhibition as a result of interaction with *S. aureus*

	Juice A	Juice B	Vegetables
Day 1	2.4	2.6	2.7
Day 3	1.5	1.6	2
Day 5	1.5	1.6	2
Day 7	0.8	0.6	1.4
Day 10	0.3	0.4	1

Anova: Single Factor				
SUMMARY				
Groups	*Count*	*Sum*	*Average*	*Variance*
Juice A	5	6.5	1.3	0.635
Juice B	5	6.8	1.36	0.788
Vegetables	5	9.1	1.82	0.422

ANOVA					
Sourceof Variation	*SS*	*Df*	*MS*	*F*	*P-value*
Between Groups	0.809333	2	0.404667	0.657995	0.535608
Within Groups	7.38	12	0.615		
Total	8.189333	14			

This table of statistical result shows the interaction of probiotics and *S. aureus*

SS means sum of squares

MS means mean squared

F means the f value

P calculated is 0.535608

P tabulated is 0.05

P cal> p tab I reject the null hypothesis that there is no significance difference in the interaction between probiotics and pathogenic bacteria.

Between groups shows how two or more groups are different. It shows how more than two groups are compared simultaneously, while within groups shows subjects who are in the same group. In calculating the *Df* (Degree of freedom) between groups, the groups are three; fermented juice A, B and fermented *S. villosum* vegetables. The formula is usually n-1 therefore that is 3-1=2. In *Df* within groups, the number of subjects in the group is 15 while the groups are 3. Therefore, that is 15-3=12.

Zones of inhibition as a result of interaction with *E. coli*			
	Fermented juice A	Fermented juice B	Fermented Vegetables
Day 1	2.8	2.7	2.6
Day 3	2.5	2.7	2.2
Day 5	1.8	1	1.6
Day 7	1	0.9	0.8
Day 10	0.5	0.4	0.2

Anova: Single Factor				
SUMMARY				
Groups	*Count*	*Sum*	*Average*	*Variance*
Fruit juice A	5	8.6	1.72	0.947
Fruit juice B	5	7.7	1.54	1.173
fermented vegetables	5	7.4	1.48	0.972

ANOVA					
Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*
Between Groups	0.156	2	0.078	0.075679	0.927553
Within Groups	12.368	12	1.030667		
Total	12.524	14			

This table of statistical result shows the interaction of probiotics and *E. coli*

SS means sum of squares

MS means mean squared

F means the f value

P calculated is 0.9227555

P tabulated is 0.05

P cal> p tab I reject my null hypothesis that there is no significance difference in the interaction between probiotics and pathogenic bacteria.

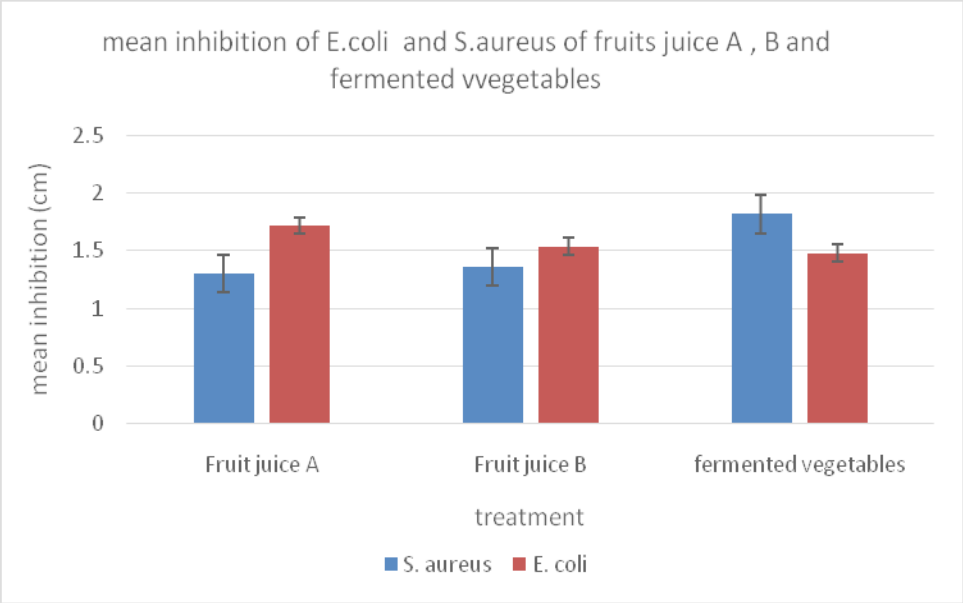
Between groups shows how two or more groups are different. It shows how more than two groups are compared simultaneously, while within groups shows subjects which are in the same group. In calculating the *Df* (Degree of freedom) between groups, the groups are three; fermented juice A, B and fermented *S. villosum* vegetables. The formula is usually n-1

therefore that is 3-1=2. In *Df* within groups, the number of subjects in the group is 15 while the groups are 3. Therefore, that is 15-3=12.

Mean of *S. aureus* and *E. coli*

	S. aureus	*E.coli*
Fermented juice A	1.3	1.72
Fermented juice B	1.36	1.54
fermented vegetables	1.82	1.48

Graph 1



This graph shows the mean of zones of inhibition of *S. aureus* and that of *E. coli.* In juice A and B, *E. coli* is inhibited most followed by *S. aureus*. In fermented vegetable, *S. aureus* has a large zone of inhibition than *E. coli*.

DISCUSSION

Fermented Juice

A great number of potential lactic acid bacteria were isolated from various naturally fermented foods, (Anandharaj and Sivasankari, 2013). The lactic acid bacteria produce lactic acid as major product from the energy yielding fermentation of sugars, (Holzapfel and Wood, 1995). The microorganisms associated with lactic fermentation of food in Africa predominantly belong to the genera of lactic acid bacteria, (Oyewole, 1997).

Figure 4.1 show *Pediococcus* bacteria which was obtained from fermented juice A; Sun-Taek Shim et al., 1990 reported that they isolated *Pediococcus* bacteria from fermented cabbage.

Figure 4.2 show *Enterococcus* bacteria isolated from fermented juice A; Tamang *et al.,* 2007 reported *Enterococcus* bacteria in fermented leafy vegetable.

Figure 4.3 show *Bifidobacteria* isolated from fermented cabbage juice. There are few reports on fermentation of vegetables found with *Bifidobacteria* cultures and P. Semjonovos *et al.,* 2014 first reported on cabbage juice fermentation with *Bifidobacteria*.

Figure 4.4 show *Bacillus* bacteria obtained from fermented juice B; fermented beetroot juice. Species of *Lactobacillus* bacteria have been isolated from fermented beetroot juice, (Panghat *et al.,* 2017) while this research show *Bacillus* bacteria was isolated from fermented beetroot juice.

Figure 4.5 show *Streptococcus* bacteria isolated from fermented juice B; fermented beetroot juice. Species of these bacteria have been isolated from fermented cabbage, (Tamang *et al.,* 2005). However, this research found *Streptococcus* bacteria was isolated from fermented beetroot juice.

Figure 4.6 show *Lactobacillus* bacteria obtained from fermented beetroot juice. The classical identification of bacterial isolates from fermented cabbage revealed that *lactobacillus* bacteria species were predominant, (Kim and Chun, 2005). Bacteria in *Lactobacillus* species are involved in spontaneous fermentation of cabbage juice, (Breidt *et al.,* 2013). However, this research found out that *Lactobacillus* bacteria were obtained from fermented beetroot juice.

Figure 4.19 and 4.20 show *Enterococcus* bacteria inhibiting the growth of pathogenic bacteria*E. coli* and *S. aureus*respectively. The zones of inhibition were measured and represented on graph 1 above as shown in the results. According to literature, cabbage juice has been shown to

have antibiotic activity against a wide range of bacteria, especially the pathogenic bacteria (Chopra and Simon, 2000). According to the results on fermented juice A on the graph, maximum zone of inhibition was observed against *E. coli* followed by*S. aureus*. These results concur with the findings of Gogo *et al.*, 2010. They reported that *E. coli* was the most resistant pathogen. The antibacterial activity of fermented cabbage juice has been reported to be due to the glucosinolates degradation byproducts found in the juice, (Saeed and Tariq 2006).

Figure 4.21 and Figure 4.22 show*Lactobacillus* bacteria from fermented juice B, fermented beetroot juice inhibiting the growth of *E. coli*and *S. aureus* respectively as shown on the inhibition zones. These zones of inhibition were measured and presented on graph 1 as shown above in the results. According to the results on fermented juice B in the graph, *E. coli* had the highest zone of inhibition than *S. aureus.*Gilliland and Speck (1977) had earlier reported that Lactobacilli showed stronger antibacterial properties against Gram-positive bacteria (*Staphylococcus aureus* and *Clostridium perfringens*) than Gram-negative bacteria (*Escherichia coli* and *Salmonella typhimurium*).

Fermented Vegetable (*S. Villosum*)

Research studies have shown that there is limited information on indigenous leafy vegetable fermentation in Africa, especially in Kenya. So far, the fermentation of leafy vegetables has not been practiced widely in Africa. A recent FAO report indicates that at global level, volumes of lost and wasted food in high income regions are higher in downstream phases of the food chain, but just the opposite in low-income regions where more food is lost and wasted in upstream phases (FAO, 2013).Fermented vegetables are potential source of probiotics as they harbor several *Lactobacillus* species and *Leuconostoc* speciesas reported by Kim and Chun, 2005.

Figure 4.7 show *Lactobacillus* bacteria obtained from fermented *S. villosum* vegetables. This bacterium has been found to be a predominant species in fermented cabbage, (Pederson *et al.,* 1969; Tamang *et al.,* 2005). Although so far there is little information on fermentation of indigenous vegetables and no information is given on probiotics in fermented *S. villosum* vegetables, *Lactobacillus* bacteria was isolated from fermented *S. villosum* vegetables in this study.

Figure 4.8 show *Leuconostoc* bacteria obtained from fermented *S. villosum* vegetables.So far there has not been a study of probiotics in fermented *S. villosum* vegetables, it has been isolated from fermented cabbage, (Steinkraus, 1997) and research studies revealed that *Leuconostoc* bacteria has been the predominant species in fermented cabbage, (Pederson and Albur, 1969). In this research, *Leuconostoc* bacteria were also isolated from fermented *S. villosum* vegetables.

Figure 4.23 and Figure 4.24 shows interaction between *Leuconostoc* bacteria acting against *E. coli*and *S. aureus* respectively. The zones of inhibition were measured and presented in graph 1 as shown in the results above. According to the results on fermented*S. villosum* vegetables obtained, *S. aureus* had the highest zone of inhibition than *E. coli.Leuconostoc* bacteria have been shown to exhibit a comparatively wider spectrum of antibacterial activity against *S.aureus* than *E. coli,* (Thakur and Roy, 2009). This coincides with the result in this study.

Biochemical Tests

Figure 4.9 show indole positive result which was in *Enterococcus* bacteria that was isolated from fermented juice A. According to literature, (Holt *et al.,* 1994) described*Enterococcus* bacteria in taxonomic scheme of Bergey's Manual of Determinative Bacteriology as indole negative.

Figure 4.10 show indole negative result was in *Pediococcus* and *Bifidobacteria,* which were isolated from fermented juice A;*Bacillus, Streptococcus* and *Lactobacillus* which were isolated from fermented juice B. Indole negative result was also in *Lactobacillus* and *Leuconostoc* bacteria which were isolated from fermented *S. villosum* vegetables. These bacteria are indole negative as classified in Bergey's Manual of Determinative Bacteriology, (Holt *et al.,* 1994).

Figure 4.11 show a positive result in methyl red test. Methyl red positive result was in *Pediococcus* species. *Pediococcus* bacteria is methyl red positive as classified in Bergey's Manual of Determinative Bacteriology, (Holt *et al.,* 1994).

Figure 4.12 show methyl red negative results which were in *Enterococcus* and *Bifidobacteria* which was isolated from fermented juice A; *Bacillus, Streptococcus* and *Lactobacillus* bacteria which were isolated from fermented juice B;*Lactobacillus* and *Leuconostoc* bacteria which were isolated from fermented *S. villosum* vegetables. These bacteria are methyl red negative as classified in Bergey's Manual of Determinative Bacteriology, (Holt *et al.,* 1994).

Figure 4.13 show citrate utilization positive test. This positive test was in *Bacillus* and *Streptococcus* bacteria that were isolated from fermented juice B. They are classified ascitrate positive in Bergey's Manual of Determinative Bacteriology, (Holt *et al.,* 1994).

Figure 4.14 show citrate utilization negative results. The negative results were in *Pediococcus, Enterococcus,* and *Bifidobacteria,* which were isolated from fermented juice A; in fermented juice B, *Lactobacillus* bacteria; *Lactobacillus* and *Leuconostoc*bacteria that were isolated from fermented*S. villosum* vegetables. They are classified as citrate negative as classified in Bergey's Manual of Determinative Bacteriology, (Holt *et al.,* 1994).

Figure 4.15 show catalase positive result. *Pediococcus*bacteria that were isolated from fermented juice A and *Bacillus* bacteria fromfermented juice B tested catalase positiveas classified in Bergey's Manual of Determinative Bacteriology, (Holt *et al.,* 1994).

Figure 4.16 show catalase negative result. Catalase negative results were in *Enterococcus* and *Bifidobacteria* which were isolated from fermented juice A; *Streptococcus* and *Lactobacillus*bacteria which were isolated from fermented juice B also tested catalase negative,*Lactobacillus* and *Leuconostoc* bacteria which were isolated from fermented *S. villosum* vegetables were also catalase negative as classified in Bergey's Manual of Determinative Bacteriology, (Holt *et al.,* 1994).

Figure 4.17 show coagulase positive result. *Streptococcus* bacteria from fermented juice B showed coagulase positive resultsas described in Bergey's Manual of Determinative Bacteriology, (Holt *et al.,* 1994).

Figure 4.18 show a negative result. Coagulase negative result were in a sample of fermented juice A*Pediococcus, Enterococcus,* and *Bifidobacteria*; *Bacillus* and *Lactobacillus* bacteria which were isolated from fermented juice B; *Lactobacillus* and *Leuconostoc* bacteria which were isolated from fermented*S. villosum* vegetables. They are classified in Bergey's Manual of Determinative Bacteriology, (Holt *et al.,* 1994) as coagulase negative.

Probiotics

Probiotics obtained from fermented juices include: *Pediococcus spp, Enterococcus spp, Bifidobacterium spp; Bacillus spp, Streptococcus spp,* and *Lactobacillus spp* while probiotics obtained from fermented *S. villosum* vegetables include *Leuconostoc spp and Lactobacillus spp.* Each of the probiotic and its health benefit is discussed below.

Pediococcus Species

Pediococcus is Gram positive coccus that is always found in pairs or tetrads. It is a homofermentative bacterium that can grow in a wide range of pH, temperature and osmotic pressure therefore being able to colonize the digestive tract,(Klaenhammer, 1993). *Pediococcus* are commonly found in fermented vegetables, (Barros*et al*., 2001). *Pediococcus* exert antagonism against other microorganisms including enteric pathogenic bacteriocins, primarily through the production of lactic acid secretion of bacteriocins known as pediocins. *Pediococcus* is known to prevent colonization of the small intestine by pathogens such as the *shigella spp, salmonella spp, E. coli and S. aureus. Pediococcus* are used in the manufacture of sauerkraut from cabbage through their ability to ferment the sugar in cabbage to lactic acid. In the animal research recently, it was reported that when administered with antibiotics, the gastrointestinal tract is disturbed. *Pediococcus* has been proved to be able to alleviate the disruptive balance of microorganisms in the gastrointestinal tract caused by antibiotic treatment and to normalize the intestinal micro flora, (Mizutani *et al*., 2007). *Pediococcus* reduces food spoilage by inhibiting pathogens and putrefactive bacteria. This strain is very resistant to destruction by stomach acids.

Health benefits of *Pediococcusspecies*

Pediococcus is able to promote a healthy inflammatory response in the intestines as well as support a healthy immune response, it functions as an immune modulator, and animals fed with *Pediococcus* have shown enhanced responses against infectious coccidoidal diseases, (Lee *et al.,* 2007). *Pediococcus* also alleviate disruptive balance of microorganisms in the gut caused by antibiotic treatment and restores the normal micro flora.

Enterococcus Species

Enterococcus is a Gram positive,lactic acid bacteria belonging to the genus *Enterococcus,* commensal bacterium inhabiting the gastrointestinal tracts of humans and other mammals. It is uniquely suited to survive the digestive process and flourish in the gut. It promotes a balanced gut environment by competing for resources that harmful organisms would otherwise consume and use to grow, possibly leading to illness, ("The genus *Enterococcus* as probiotic". Brazilian Archives of Biology and Technology, 2013).

It competes with harmful organisms for adhesion sites in the epithelial cells with pathogenic microorganisms, thus preventing the colonization and stabilization of a micro biota unfavorable to the individual. It presents features resistance to gastric juice and bile salts and therefore when administered can reach the intestine in relatively high proportions, with an additional factor in colonization. Due to these characteristics, many strains of this genus have been studied and commercialized as probiotics, (Franz *et al*., 1999). *Enterococcus* also produces a toxin known as bacteriocin that prevents the growth of other pathogenic bacteria. It has been found that culture of *Enterococcus* strain from human intestinal epithelium increased the bactericidal effects against E*. coli,* (Tarasova *et al.*, 2010).

Health benefits of *Enterococcus Species*

It boosts the immune cell function, improves regulation of cell proliferation and elevated burning capacity and prevents the colonization of pathogenic bacteria in the body of the host by competing with the pathogens for binding sites and nutrients. *Enterococcus* has also shown positive effects on diarrhea incidence, (Taras, *et al*., 2006).

Bifidobacterium Species

Bifidobacteria is a Gram positive non-motile anaerobic bacterium. They are ubiquitous inhabitants if the gastrointestinal tract, (Schell, 2002). *Bifidobacteria* are one of the major genera of bacteria that make up the colon flora in mammals; it is the dominant microorganism in the human

digestive tract of adults and infants, (Holzapfel *et al*., 2001). The dominance of *Bifidobacteria* in the human digestive tract is about 10% in adults and up to 90% in infants, (Harmsen *et al*., 2000). Research has shown that *Bifidobacterium* produces natural antibiotic substances that kill bacteria. *Bifidobacteria* is important in gut micro biota and has long been used as a probiotic to alleviate various diseases by changing the gut micro biota composition. *Bifidobacteria* has the enzymatic capabilities to break down lactose, so it can help lactose-intolerant individuals by serving as a pseudo replacement for lactase. *Bifidobacteria* initiate protective measures against pathogenic bacteria. The probiotic *Bifidobacterium* has shown metabolic capacity in gut bacteria and can increase the proportion of beneficial bacteria in the gut micro biota by cross feeding.

Health benefits of *Bifidobacterium Species*

It regulates microbial homeostasis, inhibits pathogens and harmful bacteria that colonize or infect the gut mucosa, prevents diarrhea in infants and children, *Bifidobacteria* fights *E. coli* infections, establishes initial infant micro flora, increases immunity function and produce important vitamins like B12, biotin and K2,(LeBlanc*et al*., 2013).

Bacillus Species

Bacillus is a genus of Gram positive rod-shaped bacteria that is found in the gut of human. *Bacillus* not only withstands the gastrointestinal tract, but can positively affect the composition of the micro biota. It does this by forming endospores- a durable protein envelope that protects it from heat, light and other stressors (Kenney*et al*., 2013). The best way to introduce *Bacillus* is via fermented foods such as cabbage since it contains *Bacillus* and other probiotics. Since these bacteria are spore forming, it becomes hard to eliminate them if they become opportunistic. Unless your immune system is compromised or the bacteria become opportunistic, it is a beneficial intestinal probiotic. The problem with this idea is that no one can predict when their immune system may become compromised.

Health benefits of *Bacillus Species*

Bacillus helps balance the gut, a trait it shares with other probiotics, improves abdominal pain and bloating in Irritable Bowel Syndrome (IBS) patients, (Hun, 2009).*Bacillus* also enhances immune responses.

Streptococcus Species

Streptococcus colonizes the oral cavity and upper respiratory tract of humans just a few hours after birth and establishes itself there as a predominant commensal inhabitant. *S. salivarius* is also naturally present in the upper part of the digestive tract (Qin et al. 2010), especially in the stomach and jejunum where it persists throughout the human life (Hakalehto et al. 2011; Van den Bogert et al. 2013). Live cultures of *Streptococcus* make it easier for people who are lactose intolerant to digest dairy products. The bacterium breaks down lactose, the sugar in milk that lactose-intolerant people find difficult to digest (Leboffe, 2012). This probiotic is often found in the colon and has many digestive benefits.

Health benefits of *Streptococcus Species*

Prevents oral infections and provides oral health benefits like prevention of oral cavities, produces bacteriocin-like inhibitory substances which are antimicrobial peptides. This peptide inhibits pathogenic bacteria. *Streptococcus* reduces risk of Antibiotic-Associated diarrhea, an issue that results from taking antibiotics and improves lactose digestion.

Leuconostoc Species

Leuconostoc is non-spore forming bacteria. It is catalase negative non-proteolytic organism. They are traditionally found in association with fermenting vegetables, milk and dairy products. *Leuconostoc* in general is important to fermentation of vegetables. *E. faecalis* along with *Leuconostoc mesenteroides* are the main lactic acid bacteria involved in the fermentation of Idli, a mixture of rice and black gram used in several traditional foods in Southeast Asian countries. These lactic acid bacteria are responsible for acid production, leavening of the batter and flavor formation (Nout,2009). Members of *Leuconostoc species* are very often used in production of fermented foods because of their availability to produce lactic acid. As a probiotic, *Leuconostoc* is shown to have SIgA (Secretory Immunoglobulin A) stimulating activities as well as to influence body-wide immune reactions. SIgA is the immune protein that protects mucosal surfaces such as our gastrointestinal tract from invaders like pathogenic bacteria and viruses. *Leuconostoc* provides health benefits in protecting our bodies from harmful microorganisms and keeping the intestinal cells healthier and close together by producing acids and bacteriocins, special antibacterial chemicals which reduce or eliminate pathogens.

Health benefits of *Leuconostoc Species*

Leuconostoc produces acids and bacteriocins; special antibacterial chemicals, which reduce or eliminate pathogens in the body.

Lactobacillus Species

Lactobacillus works by creating a hostile environment for the pathogenic bacteria and is often recommended as a supplement to antibiotics. *Lactobacillus* helps to promote gastrointestinal health by restoring equilibrium (Heeney et. Al., 2013). It occurs naturally in the human gut and mouth. Its name gives an indication of what it produces; lactic acid. It does this by producing an enzyme called lactase. Lactase breaks down lactose, a sugar found in milk, into lactic acid. *Lactobacillus* can be found naturally in fermented vegetables. Most commonly, *Lactobacillus acidophilus, L. casei, Bifidobacterium bifidum, B. longum* and the yeast *Saccharomyces boulardii* have been used as probiotics in humans (Payne, 1994). Probiotic bacteria could be applied

to balance disturbed intestinal microflora and related dysfunction of the gastrointestinal tract.

Health benefits of *Lactobacillus Species*

Lactobacillus supports the gut by increasing the amounts of healthy bacteria in the intestines, it helps stabilize and protect the intestinal wall and builds a healthy immune response, reduces undesirable bacteria by producing lactic acid and hydrogen peroxide, may prevent and reduce diarrhea, stabilizes the mucosal barrier and decreases intestinal permeability.

CONCLUSIONS

Based on the results of this study research, the following conclusions were made:

i. Fermented juices and fermented vegetables have bacteria that inhibit growth of pathogenic bacteria.

ii. Fermented juices inhibit growth of pathogenic bacteria most than fermented *Solanum villosum* (Black nightshade). In fermented juices, *E. coli* was inhibited most and in fermented vegetables, *S.aureus* was inhibited most.

RECOMMENDATIONS

Based on the study research, the following is recommended:

i. It is recommended that eating of fermented vegetables and drinking of fermented juices is advisable because they contain probiotic microorganisms that inhibit pathogenic bacteria.

ii. It is encouraged inclusive of the diet of fermented vegetables and fermented juices because they contain probiotics that helps to protect and restore our gut's microbes after they have been wiped out by antibiotics.

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Citation: Matasyoh G. Lexa, Karuku Judy, "Probiotic Foods and their Health Benefits", American Research Journal of Food and Nutrition, Vol 3, no. 1, 2021, pp. 1-16.