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Isolation, Characterization Of Lactic Acid Bacteria From Local Cheese And Determination Of The Acidifying Activities Of The Isolates

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ABSTRACT

Locally produced Cheese "Wara"Produced from fresh cow milk were collected from Emure-Ile in Owo, Ondo State and Subjected to microbiological analysis to determine acidifying bacteria present in it. Three genera were isolated: Lactococcus spp, Lactobacillus spp and Enterococcus spp, Lactococcus spp had the highest frequency of occurrence (50%, Lactobacillus spp (30%) while Enterococcus sp (20%). The pH of the Cheese after the fermentation processes is 4.2. The acidifying activities of the isolates were in the range of 9.008ml/ mot -34.23ml/ mol between 3rd – 24th hours of processing.

Keyword: Lactic acid bacteria, "wara," fermentation, acidifying

INTRODUCTION

Lactic acid bacteria (LAB) are groups of related bacteria that produce lactic acid as a result of Carbohydrate fermentation (Adams *et al*.; 1997). They are widespread in nature; their nutritional requirements are very complex. Hence, they predominate habitats that are rich in Carbohydrates, Protein broken down products, vitamins and environments with low oxygen which confirms their prevalence in dairy products (Stiles and Holzapfel, 1997). They are among the most important groups of microorganisms used in food fermentation. They contribute to the taste and texture of fermented products and inhibit food spoilage bacteria by producing growth inhibiting substances and large amounts of lactic acid.

These microbes are broadly used in production of fermented food products. Their growth lowers the carbohydrate content and the pH of food they ferment due to lactic acid production. It is this acidification process that is one of the most desirable effects of their growth. The lowered pH inhibit the growth of most other microorganisms including the most common human pathogens thus allowing these foods prolong shelf life. It also change the texture of the foods due to precipitation of some proteins, and the biochemical conversion involved during growth enhance the flavour of the foods (Ayad *et al*; 2004).

Production of cheese is essentially achieved by bringing four ingredients together: Milk, rennet, microorganisms and salt. The process includes the following steps: gel formation, acid production, whey expulsion, salt addition and finally ripening period. The main biochemical changes that occur in cheese manufacture is the production of lactic acid from lactose. This is achieved with different species of lactic acid bacteria (LAB).

But the responsible floras that form acid development during cheese production are starter cultures that cause decrease in the pH, formation of curd and expulsion of whey (Beresford *et al;* 2001) .The traditional Cheese – making process was developed by the nomadic Fulani and is based on the milk coagulating properties of juice from the leaves of Sodom apple (*Calotropis procera*) or pawpaw (*Carica papaya*) leaves (Aworh, 2008).

The juice obtained by crushing sodium apple leaves, is mixed with cow's milk gently heated in a hot pot over a wood fire. Following coagulation, the loose cured pieces are poured into small raffia baskets and allowed to drain (Osuntoki, 2010).

Fermentation is one of the classic methods to preserve foods. Lactic acid bacteria (LAB) and yeast are responsible for most of this fermentation (Adeleke *et al;* 2010). The weight of the microorganisms in the food is usually small, but their influence on the nature of the food especially in terms of flavour, texture and other organoleptic properties is profound (Okafor, 2009). Locally the fermentation techniques are often a small scale and on household basis, characterized by the use of simple non-sterile equipment, chance or natural inoculums, unregulated conditions, sensory fluctuation, poor durability and unattractive packaging of the processed products resulting in food of unpredictable quality (Belewu *et al*; 2005)

MATERIALS AND METHODS

Samples of fresh Cheese were bought from Fulani Camp in Emure town, few kilometers from Owo and convey to the Laboratory for analysis.

Microbiological analysis: Serial dilution was carried out using the sample and pour plate method was used. The Agar used were De Mann Roggosa Agar (MRS) Oxoid, Nutrient Agar and Nutrient Broth (Oxoid) .Enumeration and identification was carried out by total viable count while morphological characterization using colour, texture shape and size of the isolate while biochemical characterization were carried out using catalase Urease, Coagulase and sugar fermentation on the isolates for identification.

ACIDIFYING ACTIVITIES OF THE ISOLATES

Bacterial isolates were activated in MRS broth for 24hour at 30^{o}C and 0.1 ml of overnight culture was inoculated into 10 ml of sterile UHT Skim milk at 3rd, 6th 9th and 24thhour and incubated at 30^{o}C . 2ml was taken into a conical flask and 2 drops of phenolphthalein solution was added as an indicator. It was titrated against O.IM NaOH solution. Titration was terminated when the colour changes to pink and the results expressed in Milligrams per mole (Mg/mol)

RESULTS AND DISCUSSION

The total bacteria load from the sample of Cheese used ranges between I.04x10^{4} to 7.8x10^{4}CFU/ml with *Lactobacillus sp* having the highest load (7.8x10^{4}CFu/ml) as shown in Table 1 below:

Table 1: Bacteria load of local cheese 'wara'

Organisms	Bacteria load (cfu/g)
Lactococcus spp	$1.75x10^4$
Lactococcus spp	$1.60x10^4$
Lactobacillus spp	$7.8x10^4$
Enterococcus spp	$1.04x10^4$

The morphological characteristics of the isolates on the different Agar used (MRS,NA) ranges between irregular to circular in shape while it is either raised or convex in elevation and milky to yellowish in colour as shown in Table 2 below:

Table 2 Morphological characteristics of isolates.

Plates (isolation codes) Shapes Elevation Colour						
	NA	MRS	NA	MRS	NA	MRS
1 a	Irregular,	Irregular	Raised	Raised	Milky	Milky
b	Circular	Circular	Convex	Convex	Yellowish	
2 a	Irregular,	Irregular,	Raised	Raised	Milky	Milky
b	Circular	Circular	Convex	Convex	Yellowish	
3 a	Irregular	Irregular	Raised	Raised	Milky	Milky
b	Circular	Circular	Convex	Convex	Yellowish	
4 a	Irregular	Irregular	Raised	Raised	Milky	Milky
b	Circular	Circular	Convex	Convex	Yellowish	

NA=Nutrient Agar.
MRS=De Man Rogosa and Sharpe Agar.

The biochemical characterization was carried out to identify the isolates tentatively. While some are Gram+ve Rods, some are Gram+ve Cocci and all the isolate are Catalase negative. The isolates are able to ferments the sugar used (Maltose, fructose, sucrose and Lactose), as shown in Table 3 below:

Table 3: Biochemical characteristics of the isolates.

Plates	Gram rxn	Shape	Catalase	Citrate	Indole	Methylred	VP	Maltose	Lactose	Fructose	Sucrose	Probable Identities
1	+	*Cocci*	-	+	-	+	-	+	+	+	+	*Lactococcus spp*
2	+	*Rods*	-	+	-	+	-	+	+	+	+	*Lactobacillus spp*
3	+	*Cocci*	-	+	-	+	-	+	+	+	+	*Lactococcus spp*
4	+	*Cocci*	-	+	-	+	-	+	+	+	+	*Lactococcus spp*
5	+	*Cocci*	-	+	-	+	-	+	+	+	+	*Lactococcus spp*
6	+	*Rods*	-	+	-	+	-	+	+	+	+	*Lactobacillus spp*
7	+	*Cocci*	-	+	-	+	-	+	+	+	+	*Lactococcus spp*
8	+	*Rods*	-	+	-	+	-	+	+	+	+	*Lactobacillus spp*
9	+	*Cocci*	-	+	-	+	-	+	+	-	+	*Enterococcus spp*
10	+	*Cocci*	-	+	-	+	-	+	+	+	-	*Enterococcus spp*

+ve=Positive reaction
-ve=Negative ''

The frequency of the isolates also revealed that *Lactococcus sp* had the highest frequency (50%), while *Enterococcus sp* had the least frequency (20%) as shown in Table 4 below:

Table 4: Frequency of isolates

Isolates	% occurrence
Lactococcus spp	50%
Lactobacillus spp	30%
Enterococcus spp	20%

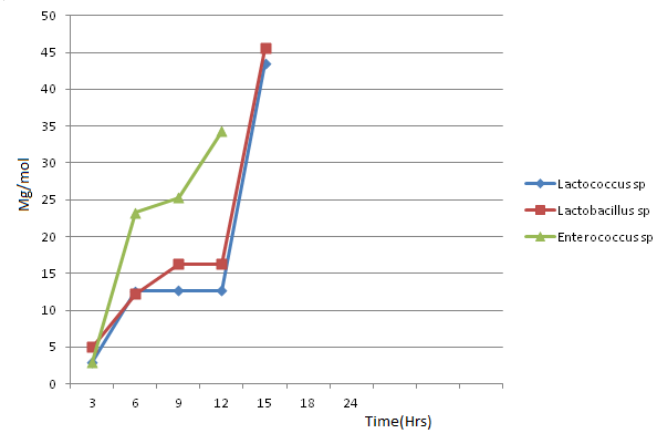
The acidifying activities of the four isolates from the 3rd hour to 24th hour of production showed that the ability to convert the Lactose in milk to Lactic acid increases gradually between 3rd to 9th hours and had reached the peak at the 24th hour as shown in Table 5 below:

Table 5: Acidifying activities of the isolates

Isolates	3rd	6th	9th	24th
Lactococcus sp	9.008ml/ mol	12.61ml/ mol	16.21ml/ mol	34.23ml/ mol
Lactococcus sp	9.008ml/ mol	12.61ml/ mol	16.21ml/ mol	34.23ml/ mol
Lactobacillus sp	10.52ml/ mol	13.75ml/ mol	19.50ml/ mol	39.65ml/ mol
Lactobacillus sp	10.52ml/ mol	13.75ml/ mol	19.50ml/ mol	39.65ml/ mol
Enterococcus sp	7.005ml/ mol	10.54ml/ mol	14.45ml/ mol	30.54ml/ mol

Each ml of the base used is equivalent to 9.008mls and therefore converted by multiplying each titre values by 9.008 mol. The acidifying activities of the isolates increases gradually as the fermentation time progresses which indicates that as the fermentation processes continues, so the rate of conversion of lactose sugar in the milk to lactic acid increases with Lactobacillus having the highest rate of conversion to lactic acid as shown in figure 1.

Figure 1:Acidifying activities of the isolates.



The results above clearly indicates that *Lactobacillus spp*, *Lactococcus spp*, and *Enterococcus spp* are the dominant lactic acid bacteria involved in the fermentation and production of local Cheese "wara" which is in agreement with the previous studies which reported high viable counts of *Lactococcus*, *Lactobacillus* and *Enterococcus spp*. in Cheese products produced from raw milk. (Centero *et al*.; 1999).

Lactobacillus sp is widely known for their ability to utilize lactose in milk to produce lactic acid which eventually lowers the pH. The ability to ferment Lactose makes *Lactobacillus spp* an important organisms which can serve as starter culture for the production of "wara". The lactic acid produced also helps to preserve the food (Marino *et al*.; 2003). *Lactococcus sp* also produces lactic acid from lactose, but at a much slower rate than *Lactobacillus sp*, which makes the latter a better starter culture. Though other metabolic products produced by *Lactococcus sp* contributes to flavour and aroma of the Cheese (Marino *et al*.; 2003). *Enterococcus sp* which has the lowest frequency contribute majorly to the development of aroma in the Cheese. However as an enteric organism may be presumed not safe, while *Lactobacillus* and *Lactococcus spp* are safe because of their non pathogenic (Garcia *et al*.;2002).

REFERENCES

1. Adams, M.R and Nicholas, L (1997).Review of the sensitivity of different food borne pathogens to fermentation. *International journal of Microbiology*, 8:227-239.
2. Belewu, M.A.; Belewu, K.Y.; Nkwunonwo, C.C (2005).Effect of biological and chemical preservatives on the shelf life of West African soft cheese. *African journal of Biotechnology*, 4:1076-1079.
3. Ayad, E.H; Nashat, S.; El-Sadek, N.; Metwaly, H and El-Soda, M. (2004).Selection of wild lactic acid bacteria isolated from traditional Egyptian dairy products according to production and technological criteria. *Food Microbiology*, London.21:715-725.
4. Beresford, T.P.; Fitzsimons, N.A.; Brenan, N.L. and Cogan, T. M (2001). Recent advances in cheese Microbiology. *International dairy journal*, 11:259-274.
5. Centeno, J.A., Menendez, S., Hermida, M., Rodriquez-Otero, J. L (1999).Main microbial floras present as natural starter in cebreiro raw Cow's milk cheese. *International journal of food Microbiology*, 33:307-313.
6. Marino, M. Maifreni, M., Rondinini, G (2003). Microbiological characterization of Montasio artisanal cheese, analysis of its indigenous lactic acid bacteria. *FEMS microbiol left*. 229: 133-140.
7. Stiles, M and Holzapfel, W (1997). Lactic acid bacteria of foods and their current taxonomy. *International journal of food Microbiology*, 36:1-29.

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