

**Mutagenesis and Selection of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* for Potential use as Starter Culture.**

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**ABSTRACT:** Mutagenesis and Selection of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* isolated from “Kindirmo” a Nigerian local yogurt for potential use as starter culture was undertaken. The mutants isolated showed ability to grow on MRS agar, and were catalase negative. Their colony color varies from white to creamy grey. Most isolates were gram positive and were presumed to have high potentials of lactic acids bacteria and few were gram negative, and were presumed to have defective gene(s). The mutant isolates showed ability to produce acids in TSB and high degree of fructose utilization. The increased acids production with increase fructose utilization by some mutant cells may also suggest the use of fructose as a supplement to increase the rate of milk fermentation in “lazy-milks”. [The Journal of American Science. 2008;4(3):80-87]. (ISSN: 1545-1003).

**Key Words:** “Kindirmo”, “Lazy-Milk”, Mutagenesis and Selection.

## INTRODUCTION

In developing countries like Nigeria, simple biotechnological techniques like mutagenesis may be adopted in order to enhance food and goods productivity. Mutation has its harmful and beneficial effects (Allan and Greenwood, 2001; Kruz, 1995; Voet, *et al.*, 1999). There are numerous documented cases where beneficial mutations with survival advantages have arisen in a population. Such beneficial mutations occur frequently among viruses, bacteria and higher organisms as well (Brown, 1992). Some of the beneficial effects are; increase in enzyme activities of mutant strain of *Leuconostoc Messenteroides* was about 2.5 fold higher than normal (Kamal, *et al.*, 2003), evolution of a single clonal line of beer yeast cells with mutations in permease and phosphatase enzymes that results in increase beer production, when yeast cells are grown in a chemostat with limited phosphate (Francis and Hansche, 1972 & 1973 and Hansche, 1975), evolution of a new metabolic pathways of metabolism of fucose and lactose by *E. coli* ( Lin and Wu, 1984 and Kenneth, 1999) and Ethidium bromide-treated *Aspergillus nidulans* cell showed significantly greater homozygotization index (HI) than controls (Becker, *et al.*, 2003). Mutagenesis has been used in the selection and improvement of Lactic acid bacteria starter culture (Harlander, 1992).

The Fulani’s (cattle rearing tribe in Nigeria) have been using their starter culture for years in “kindirmo” (local yoghurt) production with little or no attempt to improve the starter culture strains for either, to enhance product flavors or texture.

Therefore, this study has undertaken a simple biotechnology technique, mutagenesis, to see if it can improve the local starter culture strains isolates used in the production of “kindirmo” (the local yoghurt).

## **MATERIAL AND METHODS**

### **Isolation of Lactic Acid Bacterial Strains**

Lactic acid bacteria strains (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*) were isolated from “kindirmo” bought from Fulani women hawking “kindirmo” in Federal University of Technology, Yola, Nigeria. The culture was grown on MRS agar plates, (Oxoid, (2004))

### **Induction of Mutagenesis**

Mutagenesis with ethidium bromide and ultra violet (UV) light were done according to Gawel *et al.*, (2002) and Kamal *et al.*, (2003) respectively.

#### **(a). UV-Mutagenesis**

*Lactobacillus bulgaricus* and *Streptococcus thermophilus* were grown at 30°C and 42°C respectively in 100ml of tryptone soya broth (TSB) to cell optical density (O.D<sub>600</sub>) of 0.2 – 0.3. The cells were harvested by centrifugation at 5,000 X g for 15min and washed twice in 100ml cold, sterile, 0.9% NaCl solution. Portions of cell suspensions (8ml aliquots) was transferred to sterile petri dishes and radiated with UV-light (254nm) for four different periods (20, 25, 30 and 35 sec.). Each irradiated sample were centrifuged at 5,000 X g for 15min and re-suspended in 10ml TSB and incubated at 30°C and 42°C respectively for 18hr. The cultures were then diluted serially into sterile 0.9% NaCl solution and 0.1ml of serial dilution were plated on to MRS agar plates and incubated at 30°C and 42°C respectively. Then mutants were isolated

#### **(b). Ethidium Bromide Mutagenesis**

The two strains (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*) were grown to late logarithmic phase of growth in TSB. The cells were harvested and washed twice with sterile 0.9% NaCl solution and 0.5, 0.1 and 1.5g/l of ethidium bromide were added to 2ml of each cell suspension. The mixture was aerated on a shaking incubator at 30°C and 42°C for 30min respectively. The treated cells were incubated in 10ml TSB washed twice and re-suspended in 0.9% NaCl solution and after serial dilution it was spread on MRS agar plates and incubated at 30°C and 42°C respectively for 48hr. Then mutants were isolated.

### **Characterization of Mutant Isolates**

Cell morphology was performed according to Ridge (1982), catalase test according to Schieri and Blazevic (1981), physiological test was as described by Oxoid (2004) and biochemical test according to methods of Harrigan and McCance (1993), Sambrook *et al.*, (1989) and Tserovska *et al.* (2002).

## **Results**

The results of mutants isolated from UV – light mutagenesis of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* and their characterization is as shown in table 1.

All the mutants isolated showed; positive growth on MRS agar medium, negative catalase activity and all are gram positive. The mutants also showed creamy grey (CG) colony coloration with small colony size.

The cell morphology of most mutants showed rods, with only two (2) mutants (Stm 108 and Stm 109) showing cocci appearance. The mutant isolates (Lbm 102, 104, 105, 113 and 119) had scattered cell arrangement when examined with X100 oil immersion objective lens, while mutant isolate Lbm 106, had chains and scattered single cells; mutant isolate Stm 104 had single cells and scattered; and mutant isolates Stm 108 and 109 showed chains and single cells when examined with X100 oil immersion objective lens.

The mutant isolates showed three different levels of acid production in TSB. They are; positive, moderately positive and strongly positive were observed and only one mutant (Lbm 105) could not produce acid in TSB.

Positive acid production by isolates Stm 104, 108, 109; moderately positive acid production by isolates Lbm 104, 113, 119 and strongly positive acid production by isolates Lbm 102 and 106 were observed. And only one mutant (Lbm 105) could not produce acid in TSB.

The results for mutants isolated from ethidium bromide mutagenesis of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* and their characterization is shown in Table 2. All the mutants isolated showed positive growth on MRS agar medium and negative catalase activity. The colony colour of most of the mutant isolates is white except for Stm 143 and 144, which were greyish white. Colony size varies: Isolates Stm 142, 143, 144 are very small; isolates Lbm 131, Lbm 134 are small and isolates Lbm 132, 133, 140 are bigger than others. Reaction to Grams test were positive for isolates Lbm 133, 134 and Stm 140; negative for Lbm 131 and Stm 142, 143 and the Grams reaction results for Lbm 132 and Stm 143 were not determined. Mutant cell morphology showed that Lbm 131 and Stm 140, 142, 144 were cocci like in shape; isolates Lbm 133, 134 were rod like in shape and cell morphology for isolates Lbm 132 and Stm 143 were not determined. The cell arrangement when observed in X100 immersion objective lens showed that isolates Lbm 131 and Stm 144 were singles; isolates Lbm 133, 134 are scattered; isolates Stm 140, 142 are singles and scattered and that of isolates Lbm 132 and Stm 143 were not determined.

The mutant isolates showed two (2) level of acid production in TSB. The mutant isolate Lbm 132 showed strongly positive level of acid production in TSB and the rest had moderately positive level of acid production in TSB.

The result for biochemical characterization (sugar utilization) of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* mutant isolates for UV – irradiated and ethidium bromide treated strains is as shown in Table 3. The mutant isolates showed three (3) levels of sugar utilization (positive, moderately positive and strongly positive). Glucose utilization was positive among isolates Lbm 102, 104, 105, 113, 119, 132, 133 and Stm 109, 142, 143. Moderately positive by isolates Lbm 106, 131 and strongly positive by isolates Lbm 134 and Stm 108, 140, 144. Galactose utilization was positive among most isolates except Lbm 106 with moderately positive galactose utilization and Lbm 105, which showed strongly positive galactose utilization. Fructose utilization among the mutant isolates varies: Positive by isolates Lbm 132 and Stm 142; moderately positive by isolates Lbm 113, 131 and Stm 104, 140, 144 and strongly positive among isolates Lbm 102, 104, 105, 106, 119, 134 and Stm 143.

**Table 1: Characterization of UV- Irradiated *Lactobacillus bulgaricus* Mutants (Lbm) and *Streptococcus thermophilus* Mutants (Stm) Isolates.**

Isolates	Growth on MRS agar production	Catalase activity	Colony colour	Colony size	Gram reaction	Cell morphology	Cell arrangement	Acid
Lbm102	+	-	CG	Small	+	Rods	Sc	+++
Lbm104	+	-	CG	Small	+	Rods	Sc	++
Lbm105	+	-	CG	Small	+	Rods	Sc	-
Lbm106	+	-	CG	Small	+	Rods	C/Sc	+++
Lbm113	+	-	CG	Small	+	Rods	Sc	++
Lbm119	+	-	W	Small	+	Rods	Sc	++
Stm104+	-	CG	Small		+	Rods	S/Sc	+
Stm108+	-	CG	Small		+	Cocci	C/S	+
Stm109+	-	CG	Small		+	Cocci	C/S	+

Key, CG- creamy grey, CW- creamy white, W- white, Sc- scattered, S- singly, C- chain, S/C- singles and chains,

C/Sc- chains and scattered, TSB- Tryptone Soya broth, - = negative, + = positive, ++ = moderately positive, +++ = strongly positive.

**Table 2: Characterization of Ethidium bromide treated *Lactobacillus bulgaricus* Mutants (Lbm) and *Streptococcus thermophilus* Mutants (Stm) Isolates.**

Isolates	Growth on	Catalase activity	Colony colour	Colony size	Gram reaction	Cell morphology	Cell arrangement	Acid
	MRS agar production							
Lbm131	+	-	W	Small	-	Cocci	S	++
Lbm132	+	-	W	Big	ND	ND	ND	+++
Lbm133	+	-	W	Big	+	Rods	Sc	++
Lbm134	+	-	W	Small	+	Rods	Sc	++
Stm140	+	-	W	Big	+	Cocci	S/Sc	++
Stm142	+	-	W	V. small	-	Cocci	S/Sc	++
Stm143	+	-	CG	V. small	ND	ND	ND	++
Stm144	+	-	CG	V. small	-	Cocci	S	++

**Key**, CG- creamy grey, W- white, S- singles, C- chains, Sc- scattered, S/Sc singles and scattered, V. small- very small,

TSB- Tryptone soya broth, ND- not detectable, + = positive, ++ = moderately positive, +++ = strongly positive, - = negative.

**Table 3: Biochemical Characterization of *Lactobacillus bulgaricus* Mutants (Lbm) and *Streptococcus thermophilus* Mutants (Stm) Isolates from UV – Irradiated and Ethidium Bromide-Treated Strains.**

Isolates	Treatment	Glucose	Galactose	Fructose
Lbm102	UV-25sec	+	+	+++
Lbm104	UV-35sec	+	+	+++
Lbm105	UV-35sec	+	+++	+++
Lbm106	UV-35sec	++	++	+++
Lbm113	UV-35sec	+	+	++
Lbm119	UV-35sec	+	+	+++
Lbm131	0.5g/dm <sup>3</sup>	++	+	++
Lbm132	0.5g/dm <sup>3</sup>	+	+	+
Lbm133	0.5g/dm <sup>3</sup>	+	+	++
Lbm134	1.0g/dm <sup>3</sup>	+++	+	+++

Stm104	UV-35sec	++	+	++
Stm108	UV-35sec	+++	+	+
Stm109	UV-35sec	+	+	+
Stm140	1.0g/dm <sup>3</sup>	+++	+	++
Stm142	1.0g/dm <sup>3</sup>	+	+	+
Stm143	1.5g/dm <sup>3</sup>	+	+	+++
Stm144	1.5g/dm <sup>3</sup>	+++	+	++

**Key**, + = positive, ++ = moderately positive, +++ = strongly positive

## Discussion

The ability of mutant isolates to grow on MRS agar medium and are catalase negative is an indication that the mutant isolates still retain biological activities of wild type strain (Oxoid, 2004; Togo, 2002). The isolates that are Gram positive are presumed to have potentials of lactic acid bacteria and the rest could be mutants with defective gene. Lactic acid bacteria have been consistently demonstrated to be responsible for the lactic acid fermentation of milk (Brock and Madigan, 1991; Prescott *et al.*, 1999) and non-dairy materials (Steinkraus, 1996; Varnam, 2002). The mutant isolates showed ability to produce lactic acid in TSB and fructose utilization, which is in line with the work of Buchanan and Gibbons (1974). The increased fructose utilization observed may also explain the increased acid production in some mutant cells and this may likely suggest the use of fructose as supplement to increase the rate of milk fermentation in “lazy – milk” similar to research work of Igyor (2005) where he used yeast extract supplementation in milk fermentation.

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