

# Isolation Identification and Characterization of Cyanide Tolerating LAB from Fermented Cassava Pulp Juice

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## ABSTRACT

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Lactic acid bacteria are predominant microorganisms that play important roles during cassava fermentation. Most efforts to develop cyanide tolerant isolates for cassava fermented products have been unsuccessful because of cyanide toxicity in cassava tubers. The objectives of this study was identify cyanide tolerating bacteria and study their tolerability efficiency. 119 lactic acid bacteria were isolated from fermented cassava pulp juice. Out of the total isolated bacteria, ten potential cyanide tolerating bacteria was screened. The cyanide tolerance efficiency was studied at 100, 400, 600 and 8000mg/L potassium cyanide, growth at different temperature and pH were analyzed. The maximum growth rate reading of the screened isolates ranges (0.674 to 1.116) at 600nm in maximum cyanide concentration. Isolate CD1, LA2, LA1 and CD2 showed maximum cyanide tolerance of (74.4, 72.66, 70.66 and 68.73%) respectively at 800mg/l after 48hr of incubation. The cyanide tolerability potential of individual isolates varies across different cyanide concentration. Above all 10 isolates were resistant to maximum cyanide concentration and possess potential of cyanide tolerating properties that help their selection and application in a controlled process to detoxify cassava roots for food and feed utilization. There was a general decrement in all samples over 48h fermentation period from 6.00 to 3.18 and the bacterial isolates showed significant growth at room temperature which were between 25 and 40 °C.

**Keywords :** Cassava, Fermentation, pulp juice, HCN Tolerance

## I. INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is the third agricultural resource after rice and maize as a source of calories in tropical countries (De Oliveira et al., 2015). In Africa, tendency for cassava's use is almost 40%, and represents nearly twice of that of the world (Tetchi et al., 2012). For 200 million people (more

than a quarter of the continent's total population), cassava represents staple food necessities of which each consumes more than 100 kg per year (Pierre, 2012). Cassava is traditionally processed into a wide variety of fermented products such as atteki gari, lafun, fufu, and manioc or chips, particularly suited to transportation, trade and rapid preparation of meals (Kouamé et al., 2012). Through thousands of years,

demand for the production and consumption of fermented foods has extremely increased and accordingly, those foods occupied a substantial part of the diet worldwide (Elyas et al., 2015).

Cassava toxicity in humans is a well documented problem. Cassava tubers vary widely in their cyanogen content, although most varieties contain 15 to 400 mg HCN per kg fresh weigh (Padmaja, 1995). Cyanide doses of 50 to 100 mg are reportedly lethal to adults. Several diseases are associated with the consumption of inadequately processed cassava roots, such as tropical ataxic neuropathy, endemic goiter and spastic Paraparesis (Konzo) (Siritunga ,2002). Konzo is mainly a disease of women and children. It is an acute disease, rapidly and permanently crippling the victim by damaging nerve tracts in the spinal cord that transmit signals for movement, causing a spastic paralysis of both legs (Howlett, 1999).

Hydrocyanic acid is a highly toxic compound for humans. Therefore, the toxic potential of cassava roots should be addressed during cassava root processing before human consumption. The roots have to be detoxified to less than 10 ppm, which is the safe limit proposed by the World Health Organization (WHO, 1991). The lactic acid fermentation is one of the most used processes for cassava detoxification during the fermentation process the formation of lactic acid decreases the pH below 6. This needs microorganisms which tolerate and detoxify cassava cyanides.

Some of the common microorganisms involved in cassava fermentations are lactic acid bacteria. These bacteria have antagonistic properties against food damaging microorganisms, such as *Styphylococcus aureus* *Salmonella*. It also detoxify the ant nutritional factors due to their metabolic pathways. Some of the lactic acid bacteria tolerate cassava cyanide to certain level of concentration. (Buckle, 1985).

LAB generally regarded as safe (GRAS), play an essential role in the majority of food fermentations and preservation, and a wide variety of strains are routinely employed as starter cultures in the manufacture of dairy, meat, vegetable, and bakery products (Elyas et al., 2015). They contribute to the enhancement of sensory, quality and safety features of these fermented foods (Holzapfel and Wood, 2014). Their antimicrobial activity has been attributed to produced metabolites such as organic acids, carbondioxide, hydrogen peroxide, diacetyl and bacteriocins which can inhibit pathogenic and spoilage microorganisms, extending the shelf life and enhancing the safety of food products (Piard and Desmazeaud, 1992).

Remarkable improvement in cassava fermentation was achieved by the use of pure cultures of cyanide tolerating microorganisms involved in the natural fermentation for solid state cassava mash (Ahaotu et al., 2011). Currently examining the cyanide tolerability potential lactic acid bacteria from fermented cassava pulp juice involved in the natural fermentation process is an important.

For these reasons, the objective of this study was to isolate and identify LAB which have a cyanide tolerating potential for its role in detoxification of cyanide present in cassava tuber fermentation for food use.

## II. MATERIALS AND METHODS

### Sample collection

Cassava tuber was collected from different geographical location of southern part of Ethiopia, from a model farmer who cultivates white cassava varieties. Whole fresh tuber was first washed, cleaned, and softened, then peeled the pulp. The pulp was then knife-chopped into small, manageable slices. This cassava pulp was grated and pressed in a fine

cloth to obtain pulp juice. The juice was allowed to ferment for 72hrs at ambient temperature (26 -32°C).

Out of the fermented pulp juice isolation of bacteria using De Mann Rogosa and Sharpe (MRS) media was carried out using serial dilution. The serial dilution was carried out up to 10<sup>4</sup>- 10<sup>6</sup> dilution factor; 0.1ml of the diluent was aseptically dispensed into dishes pour plated with already sterilized molten media anaerobically for 24 to 48hrs at 30°C. The pure cultures were kept on slant and stored in refrigerator at 4°C.

Temperature tolerance test:

Heat tolerance was determined using a Rajkowska modified method (Rajkowska et al., 2010). Growth at different temperatures was estimated by inoculating 10<sup>6</sup> cfu/mL of activated culture into MRS liquid medium. The samples were incubated at four different temperatures ranging from 25 to 50 °C for 24h. Bacterial growth was measured using a spectrophotometer by reading the optical density at 600 nm (OD<sub>600</sub>) against un inoculated broth as the blank.

Screening isolates for their resistance to hydrogen cyanide

Test tubes each containing 5mL of screening medium (glucose, 2% (w/v) in 100 mL of distilled water) were autoclaved at 121°C for 15 minutes. Then, aliquots (0.1 mL) of KCN solution (800 mg L<sup>-1</sup>) sterilized by tyndallisation according to (Barrow and Feltham., 1999) was added into each test tube containing the screening medium. Each bacterial isolate was inoculated into each test tube. The test tubes were incubated at room temperature (30±2 °C) for 2 days. The sensitivity or resistance of each isolate to cyanide was monitored with a spectrophotometer at 600 nm against a distilled water blank.

### III. RESULTS AND DISCUSSION

Isolation and Identification of Microorganisms Growing in the Presence of Cyanide.

Isolation of microorganisms from fermenting cassava pulp juice results in tables 4.1 show the identities and the microorganisms were identified by comparing their cultural, physiological and biochemical characteristics. A total of 132 bacterial isolates were recovered from fermented pulp juice. These bacteria were tested for their ability to tolerate high concentrations of cyanide. Twenty isolates showed good growth on media supplemented with maximum cyanide concentration. Out of the twenty potential isolates, ten isolates (CD1, LA2, LA1, CD2, LA5, CD5, LA6, CD4, LA4 and CD6) were selected and purified based on their resistance to maximum concentration of cyanide (800mg/l).

Effect of PH on tolerability potential of bacterial isolates.

During the 48h of fermentation, pH decrement was observed in all cassava pulp samples (Table 1). The pH ranged from 6.00 to 6.20 at the beginning of the fermentation process (0h) while at the end of 48h retting the pH had significantly changed to between 4.00 and 3.18. There was a general decrement in all samples over 48h fermentation period the stability and safety of foods in cassava fermentations (Caplice and Fitzgerald, 1999). In the same way (Kostinek et al., 2007 and Edward et al., 2012), also suggested that cyanide tolerating isolates were rapid acid producer's and minimize the food spoilage during cassava fermentation. This is because spoilage bacteria, as well as pathogens, notably those including members of Enterobacteriaceae family, do not grow below this pH (4.2) level.

Table 2 : Changes in pH of fermenting cassava pulp juice samples

Time (hr)	pH									
	Isolates									
	CD <sub>1</sub>	LA <sub>2</sub>	LA <sub>1</sub>	CD <sub>2</sub>	LA <sub>5</sub>	CD <sub>5</sub>	LA <sub>6</sub>	CD <sub>4</sub>	LA <sub>4</sub>	CD <sub>6</sub>
0h	6.17	6.00	6.08	6.11	6.00	6.09	6.21	6.20	6.06	6.03
24	4.1	4.03	4.00	4.07	4.01	4.21	4.04	4.04	4.16	4.13
36	3.91	3.88	3.71	3.97	3.82	3.79	3.70	3.88	3.91	3.80
48	3.25	3.18	3.33	3.41	3.52	3.29	3.59	3.44	3.20	3.33

### Temperature tolerance

The heat tolerance patterns of ten isolated bacterial cultures were presented in Table 3. All isolates gave absorbance (OD<sub>600</sub>) reading above 0.5 when subjected to grow up to 45 °C after 24hr of incubation however only two of the bacterial isolates namely (CD<sub>1</sub> and LA<sub>5</sub>) showed good growth. There was no significant change on growth pattern of the remaining isolates. In similar way when the isolates subjected to grow at 55°C and incubated for 24hrs, only isolate LA<sub>2</sub>, LA<sub>5</sub>, CD<sub>4</sub> and CD<sub>6</sub>, gave absorbance (OD<sub>600</sub>) readings above 0.500. This result indicates that most of the bacterial isolates cannot survive and died at high temperature. On the other hand, most of the isolated bacterial cultures showed significant growth at room temperature which were between 25 and 40 °C, and the above mentioned isolates ( CD<sub>1</sub> and LA<sub>5</sub>) showed higher growth rate at human body temperature which were between 35°C and 40 °C.

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Table 3 show the growth tolerance of bacterial isolates at different temperature

No	Isolate Code	OD value (600)		
		35°C	45°C	55°C
1	CD <sub>1</sub>	0.500	0.945	0.647
2	LA <sub>2</sub>	0.505	0.522	0.507
3	LA <sub>1</sub>	0.538	0.555	0.499
4	CD <sub>2</sub>	0.522	0.528	0.494
5	LA <sub>5</sub>	0.505	0.749	0.599
6	CD <sub>5</sub>	0.506	0.545	0.497
7	LA <sub>6</sub>	0.502	0.535	0.498
8	CD <sub>4</sub>	0.501	0.524	0.508
9	LA <sub>4</sub>	0.513	0.534	0.498
10	CD <sub>6</sub>	0.504	0.534	0.509

### Morphological and biochemical characteristics of bacterial isolates

The morphological features of the colonies on MRS agar plates are presented in Table 3. Colonies obtained were seen to be rod and

cocci in shaped and chain arrangements. Most of the isolates were gram positive, motile and catalase

Serial No	Isolate	OD value at 600nm	% of Tolerances
1	CD <sub>1</sub>	1.116	74.40
2	LA <sub>2</sub>	1.099	72.66
3	LA <sub>1</sub>	1.031	68.73
4	CD <sub>2</sub>	1.006	70.66
5	LA <sub>5</sub>	0.830	55.33
6	CD <sub>5</sub>	0.804	53.33
7	LA <sub>6</sub>	0.780	52.00
8	CD <sub>4</sub>	0.716	47.73
9	LA <sub>4</sub>	0.696	46.00
10	CD <sub>6</sub>	0.674	44.93

e and catalase positive.

**Table 4** Biochemical and morphological characterization of tolerant bacterial isolates.

*Note positive (+), negative (-), not determined (ND).*

**Screening isolates for their resistance to hydrogen cyanide**

From the selected individual isolates, ten of the bacterial isolates were tolerated all the four different KCN concentrations whereas the tolerability potential of the remaining isolates decreased when the KCN concentration exceeds above 600mg/l and showed less growth and tolerance than the above mentioned isolates. The tolerability potential of isolate CD<sub>1</sub>, LA<sub>2</sub>, CD<sub>2</sub> LA<sub>1</sub> and LA<sub>5</sub> to cyanide concentrations of about 800mg/l was (74.4, 72.66, 70.66, 68.73 and 55.33%) respectively. This resistance property is responsible for the dominance of lactic acid bacteria in natural micr

N	Isolates code	Cell shape	Cell arrangement	Gram reaction	Catalase	Motility
1	CD <sub>1</sub>	Rod	Chain	+	-	+
2	LA <sub>2</sub>	Rod	Chain	+	-	+
3	LA <sub>1</sub>	Rod	Chain	+	-	+
4	CD <sub>2</sub>	Rod	Chain	+	-	+
5	LA <sub>5</sub>	Cocci	Chain	+	+	+
6	CD <sub>5</sub>	Rod	Chain	ND	+	+
7	LA <sub>6</sub>	Cocci	Chain	-	-	-
8	CD <sub>4</sub>	Cocci	Tetrad	+	-	+
9	LA <sub>4</sub>	Rod	Chain	+	-	+
10	CD <sub>6</sub>	Rod	Tetrad	-	+	-

oflora of fermented cassava pulp juice and were well adapted to the contents of cyanide present in cassava fermentation. While the tolerance of isolate CD<sub>5</sub>, LA<sub>6</sub>, CD<sub>4</sub>, LA<sub>4</sub> and CD<sub>6</sub> ranges in between (53.60 to 44.93%). This finding shows that CD<sub>1</sub>, LA<sub>2</sub> and CD<sub>2</sub> were found to be more efficient than the isolates mentioned above at tolerating maximum cyanide concentration at alkaline condition well above pKa value of cyanide. (Table 5).

Table 5 shows the isolate with its respective optical density values and its tolerability potential

**IV. CONCLUSION**

It is well known that biological treatment of cyanide by utilizing indigenous microorganisms can be less expensive and more environmentally friendly for cyanide removal from cassava fermentation compared with conventional techniques for instance chemical methods. The present study primarily focused on the isolation and purification of the microorganism

ms which tolerate cyanide and use as the sole source of nitrogen in alkaline conditions. The isolates CD<sub>1</sub>, LA<sub>2</sub> LA<sub>1</sub>, and CD<sub>2</sub> on basis of the higher efficiency of cyanide tolerance of (74.4, 72.66, 70.77 and 68.73%) respectively after 48hr of incubation. The isolates growth was maximum at pH 4.0 to 3.18, and tolerated cyanide concentrations of up to 800mg/l which makes it a good candidate for the biological detoxification of hydrogen cyanide in cassava based foods.

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Conflict of Interest.

Authors declare that it has no conflict of interest.

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