Linamarase production by some microbial isolates and a comparison of the rate of degradation of cassava cyanide by microbial and cassava linamarases

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Abstract

Production of linamarase and the effects of media composition on enzyme production were studied. A total of eight linamarase-producing bacteria were isolated from fermenting cassava tubers and soil samples. Selection of the isolates was based on their fast growth in media containing 800 mg/L potassium cyanide solution. Eight of the isolates which showed very fast growth in the growth medium as demonstrated by increase in their optical density readings to at least 0.6 in the cyanide containing media were selected for further studies. The isolates were identified as *Lactobacillus plantarum, Lactobacillus fermentum, Lactobacillus amylovorus, Lactobacillus cellobiosus, Leuconostoc mesenteroides, Pseudomonas stutzeri, Bacillus pumilus* and *Bacillus subtilis.* All the isolates were grown in media containing Tween 80 solution and in control media without the surfactant. Best enzyme activity of 6.82 U/mL was obtained in the medium containing Tween 80 solution and *Lactobacillus fermentum* as the test bacterium. Comparatively, linamarase production by the isolates in media without Tween 80 showed lower enzyme productivity. Cassava endogenous and microbial enzymes were tested for their abilities to hydrolyze cyanide in cassava flour samples pretreated to either remove the endogenous or microbial enzyme. Residual cyanide in cassava flour samples treated with linamarase of *Lactobacillus plantarum* was undetected in 30 h, while in contrast, the residual cyanide in cassava flour samples treated with endogenous linamarase was 0.39 mg/10 g cassava flour after 80 h. Residual cyanide in the untreated control sample was 1.98 mg HCN /10 g cassava flour after 80 h. The results from this finding demonstrated improved cassava cyanide degradation with microbial linamarase as compared to endogenous cassava linamarase. Massive inoculation of fermenting cassava tubers with the isolates reported in this study would enable better control of the cassava fermentation process and may lead to the production of standardized and non-toxic cassava food products.

Keywords: linamarase, cassava cyanide reduction, microorganisms, surfactant.

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Cassava, the vital food in the tropical areas of Africa, Asia and Latin America is the third most important source of calories in the tropics after rice and corn [1]. Cassava is toxic as it contains large amounts of cyanogenic glucosides, linamarin and lotaustralin [2–4]. Daily consumption of foodstuffs which still contain residual levels of these cyanogenic compounds can result in chronic diseases such as goitre, cretinism, tropical atoxic neuropathy and tropical diabetes [5,6].

Cassava processing allows the reduction of toxic endogenous cyanogens which are present in variable concentrations in cassava tubers by volatilization of HCN present in the cassava tubers and consequent reduction of the cyanide levels. During the consequent fermentation, roots are softened, the disintegration of the tissue structures result in the contact of linamarin with linamarase and subsequent hydrolysis to glucose and cyanohydrins which easily break down to ketone and hydrocyanic acid [7]. Retting is one of the simplest methods for the processing of cassava tubers into various African staple foods. In some conditions, retting may take considerably longer periods particularly with tubers older than 24 months and some of the tubers steeped under this condition may fail to soften. Other processing techniques such as cooking, sun-drying, oven-drying and roasting have been developed in different parts of the world to reduce the cyanide content of cassava-based foods to an acceptable level. However none has achieved a complete detoxification [8]. The rate of fermentation depends on the processing method, size of cut tubers and the age of roots. With the increasing demand for foods due to a rapid population growth in many developing countries, the need has arisen to reduce the process time for retting of cassava roots.

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Linamarase, β-D-glucosidase (EC.3.2.1.21) is an enzyme that converts cassava cyanide to hydrogen cyanide (HCN) that either dissolves readily in water or is released into the air. They constitute a group of well studied hydrolases that have been isolated from members of all three domains of life, *i.e.*, eukaryotes, bacteria and archae. The principal reaction catalysed by this class of enzymes is the hydrolytic cleavage of β-glucosidic linkages of low molecular mass glucosides [9]. Apart from hydrolysis, β-glucosidases can catalyse reverse hydrolysis, giving glucose disaccharides and trisaccharides as products when using the glucose as substrate. Linamarase, is found in the leaves and roots of plants such as cassava, lima beans and flax, and are also produced by microorganisms. When the cellular structure of cyanogenic plants is disrupted, the intracellular glucoside becomes exposed to the extracellular enzymes linamarase. Linamarin, is first hydrolysed by linamarase to produce β-D-glucopyranose and 2-hydroxyisolentyronotrite or acetone-cyanohydrin, after which the latter is degraded to acetone and hydrogen cyanide (Fig. 1).

The aim of the present work is to comparatively study the effect of some media on the production of microbial linamarase and an assessment of the rate of cassava cyanide degradation by linamarase from microbial and cassava sources.

MATERIALS AND METHODS

Chemicals and reagents

Potassium cyanide (KCN), magnesium chloride ($MgCl_2 \cdot 2H_2O$), sodium carbonate (Na_2CO_3), potassium chloride (KCl), calcium chloride ($CaCl_2$), ferric sulphate ($FeSO_4 \cdot 7H_2O$), magnesium sulphate ($MgSO_4 \cdot 7H_2O$), zinc sulphate ($ZnSO_4$), mercuric chloride ($HgCl_2$), linamarin, ammonium sulphate $(NH_4)_2SO_4$), manufacturer: BDH Chemicals Ltd., Poole, England. Sodium dihydrogen phosphate (NaH_2PO_4), sodium hydrogen phosphate (Na_2HPO_4), potassium hydrogen phosphate (K_2HPO_4), sodium chloride (NaCl), manufacturer: Sigma-Aldrich Chemie Gmbh, Steinheim, Germany. Cupper sulphate ($CuSO_4$ x $5H_2O$), manganese sulphate ($MnSO_4 \cdot 5H_2O$), manufacturer: Bio-Lab UK Ltd., Cockspur Street, London. Sodium hydroxide (NaOH) manufacturer, Nexill World Chem Chadwell Health, Essex England. Potassium hydroxide (KOH) manufacturer: Avondale Laboratories, Banbury, Oxon, England. Picric acid, manufacturer: Qualikems Fine Chemicals PVT. Ltd., India. Glucose manufacturer: May and Baker Ltd., Dagenham, England. Nutrient agar, MRS agar, yeast extract, peptone, manufacturer: Oxoid, Ltd. UK. Tween 80, manufacturer, Difco Laboratory, USA.

Isolation of microorganisms from fermenting cassava tubers and soil samples

Fermenting cassava tuber (*ca.* 10 g) was aseptically removed, homogenized in distilled water in a sterile blender and stirred with a sterile glass rod. Then, 1 mL of the homogenate was withdrawn with a sterile pipette into 9 mL 0.1% peptone water diluents and mixed. About 0.1 mL was aseptically withdrawn with a sterile pipette and inoculated onto Nutrient agar for the isolation of bacteria and MRS agar for the isolation of lactic acid bacteria. The plates were incubated for for 24 h at 35 °C.

Soil samples (*ca.* 20 g) were collected from a cassava processing mill and 1 g of soil was added into a conical flask containing 10 mL of distilled water. The mixture was thoroughly shaken and 1 mL was serially diluted in normal saline diluents. The dilutions (0.1 mL) were plated out on Nutrient agar plates and incubated for 24 h at 35 °C. Pure cultures of the isolates were obtained by streaking onto fresh agar plates. The cultures were given arbitrary codes and stored at 4 °C.

Preparation of modified local media

Banana broth (BB) – peeled ripe banana fruit (200 g) was homogenized with mortar and pestle in one liter of distilled water and filtered with a stainless steel mesh.

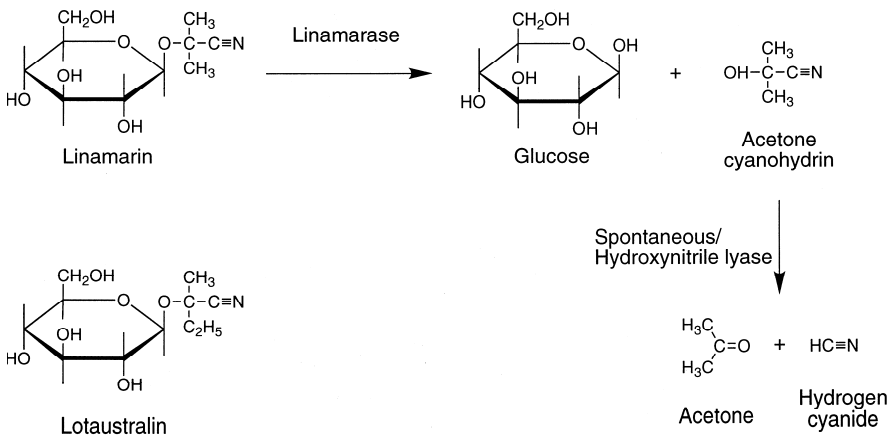

Figure 1. Breakdown of cyanogenic glucosides of cassava by linamarase.

The filtrate was re-filtered with a Whatman No. 1 filter paper. The broth was fortified with 0.1% ammonium sulphate and sterilized by autoclaving at 121 °C for 10 min.

Orange broth (OB) – fully-ripped oranges (200 g) were peeled and ground with a Corona mill (Medellin, Colombia) after removing the seeds in one liter of distilled water, and filtered with a stainless steel mesh. The filtrate was re-filtered with a Whatman No. 1 filter and fortified with 0.1% ammonium sulphate and sterilized by autoclaving at 121 °C for 10 min.

Tomatoj broth (TJB) – fresh tomato fruits (200 g) were homogenized in distilled water and filtered with a stainless steel mesh. The filtrate was re-filtered with a Whatman No. 1 filter paper and made up to 100 ml with distilled water. The broth was fortified with 0.1% ammonium sulphate and autoclaved at 121 °C for 10 min.

Screening the isolates for their resistance to cyanide

Test tubes each containing 5 mL of screening medium (yeasts extract, 0.5%; peptone 0.7%, glucose, 2% in 100 mL of distilled water) were autoclaved at 121 °C for 15 min. Then, aliquots (0.1 mL), potassium cyanide (KCN) solution (800 mg/L) which was sterilized by tyndallization according to Collins and Lyne [10] was added into each test tube containing the screening medium. A loopful of 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/ resistance of each isolate to cyanide were monitored with a Spectrumlab 23A spectrophotometer at 600 nm against distilled water blank. Isolates that gave OD readings of at least 0.6 after 2 d incubation period were selected for further work. The isolates were identified respectively as *Lactobacillus plantarum, Lactobacillus fermentum, Lactobacillus amylovorus, Lactobacillus cellobiosus, Leuconostoc mesenteroides, Pseudomonas stutzeri, Bacillus pumilus* and *Bacillus subtilis* based on the taxonomic descriptions given by Holt *et al.* [11] and Skinner and Lovelock [12].

Development of inocula of lactic acid bacterial isolates. Inoculum was prepared from a stock culture by transferring to an Erlenmeyer flask (250 ml) containing 100 ml of medium described by Okafor and Ejiofor [13]: NaCl, 0.3%; $(NH_4)_2SO_4$, 0.1%; KH_2PO_4, 0.05%; $MgSO_4$, 0.02%; $CaCl_2$, 0.02%; lactose, 3%; linamarin, 0.15%. The medium was sterilized at 121 °C for 15 min. The inoculum was grown for 24 h in a Gallenkamp orbital incubator at 50*g* at 30 °C. The cells were collected by centrifugation at 2515*g* for 15 min in a Gallenkamp junior centrifuge, washed and diluted with sterile distilled water to an optical density of 0.1 measured in a Spectrumlab 23A spectrophotometer at 600 nm.

Development of inoculum of Pseudomonas sutzeri. The medium for multiplication of *Pseudomonas sutzeri* had the following composition per litre of distilled water: K_2HPO_4, 1 g; $MgSO_4 \cdot 7H_2O$, 0.2 g; $CaCl_2$, 0.01 g; NaCl, 0.01 g; $MnSO_4$, 2 mg; $CuSO_4 \cdot 5H_2O$, 0.2 mg; $ZnSO_4$, 0.2 mg; glucose, 2 g; tryptone, 1 g; and linamarin, 3 g. The medium was sterilized at 121 °C for 15 min. The inoculum was grown for 24 h in a Gallenkamp orbital incubator at 50*g* at 30 °C. The cells were collected by centrifugation at 2515*g* for 15 min, washed and diluted with sterile distilled water to an optical density of 0.1 measured in a Spectrumlab 23A spectrophotometer at 600 nm.

Development of inocula of Bacillus pumilus and Bacillus subtilis. The medium contained the following per litre: linamarin 3 g; peptone, 10 g; KCl, 1.0 g; $MgCl_2 \cdot 2H_2O$, 0.2 g; $CaCl_2 \cdot 2H_2O$, 0.25 g; $FeSO_4 \cdot 7H_2O$, 5 mg; $MnSO_4 \cdot 5H_2O$, 1 mg; Na_2HPO_4 5.26 g; NaH_2PO_4 5.4 g. The medium was sterilized at 121 °C for 15 min. The inoculum was grown for 24 h in a Gallenkamp orbital incubator at 50*g* at 30 °C. The cells were collected by centrifugation at 2515*g* for 15 min, washed and diluted with sterile distilled water to an optical density of 0.1 measured in a Spectrumlab 23A spectrophotometer at 600 nm.

Cultivation of bacteria

Lactic acid bacterial isolates were grown in the following media: OB, TJB, BB and MRS broth. *Pseudomonas stutzeri*, *Bacillus pumilus* and *Bacillus subtilis* were cultured in OB, TJB, BB and Nutrient broth. The isolates were added into 100 mL media containing 2% linamarin solution and incubated at 32±2 °C for 24 h. At the end of the incubation period, the culture was centrifuged at 2515*g* for 10 min. The effects of Tween 80 on the production and activity of the linamarase were studied by adding 0.1% Tween 80 into each duplicate culture flasks at the time of incubation. At the end of the incubation period, the culture was centrifuged at 2515*g* for 10 min.

Bacterial enzyme extraction

The harvested cells were washed with 0.2 M phosphate buffer (pH 6.5) and the washed cell suspension was disrupted by sonication for 10 min. using Biologics Ultrasonic homogenizer, model 150VT (115V/60Hz). Following disruption, the mixture was centrifuged at 2515*g* for 15 min. The supernatants of the two centrifugations were combined and suspended in 0.2 M phosphate buffer (pH 6.5). Ammonium sulphate was added with stirring to 40% saturation followed by centrifugation at 2515*g* for 10 min. Linamarase activity was then determined.

Extraction of endogenous cassava linamarase enzyme. Fresh cassava peel (100 g) was homogenized in 300 mL 0.2 M phosphate buffer (pH 6.5) using a mortar and pestle chilled by ice. The homogenate was centrifuged at 2515*g* for 15 min. The supernatant was

applied to a column of Sephadex G-25 (7 cm×62 cm) pre equilibrated with phosphate buffer (pH 6.5) and eluted with the same buffer to remove lower molecular weight compounds. Fractions were collected from the column and measured for linamarase activity.

Comparison of the rate of detoxification of cassava flour by microbial and endogenous cassava linamarases

Cassava tubers (NR 8082) were employed for this assay. The cassava tubers were peeled with knife and washed with tap water. The tubers were cut into cubes of approximately 10 cm and sun dried. The dried cassava tubers were milled with a mechanical greater.

To determine the effects of endogenous linamarase activity on the cyanogenic glucoside of cassava, cassava flour (10 g) contained in glass bottles was sterilized with 0.1% $HgCl_2$ solution for 5 min, rinsed with distilled water and finally treated with 1% of hypochlorite solution for 5 min. To wash off the effects of these chemicals, the cassava flour was finally rinsed for 15 min in sterile distilled water.

To assess the role of microbial fermentation on the degradation of the cyanogenic glucoside of cassava, 1, 5-gluconolactone (an inhibitor of endogenous glucosidase enzyme) was added to cassava flour at 10% concentration [14]. The effect of the inhibitor was removed by treating the cassava pulp with 50% ethanol solution and rinsing with sterile distilled water.

Buffered linamarase enzymes of both microbial and cassava origin (20 mL each) were added into 10 g of treated cassava flour contained in glass bottles. Samples of untreated and uninoculated cassava flour (10 g) served as control. Then 2 mL of 2% KOH and 1mL of picric acid:Na_2CO_3:H_2O, 1 mL:5 g:200 mL, contained in test tube were suspended in each bottle just before the bottles were sealed. The system was incubated at intervals of 10 h for 80 h at 30 °C. The HCN liberated from the cassava flour was absorbed by the alkaline picrate solution in the test tube [14]. After incubation, the reaction was stopped by placing the bottles in iced water. The colour that developed was read at 510 nm. Cyanide levels were extrapolated from KCN standard curve.

Analyses

Cyanide was determined by a modification of the alkaline picric acid method of Williams and Edwards [15] as follows: various quantities of standard (50, 100, 150 and 200 μg/ml) solution of KCN were added into tubes containing 2 ml of 2% KOH and 1ml of picric acid:Na_2CO_3:H_2O, 1 mL:5 g:200 mL. The tubes were incubated for 10 min in a 37 °C water bath, cooled for 20 min in a refrigerator and read in a Spectrum lab 23A spectrophotometer at 510 nm. The readings were used to draw a standard curve for micrograms KCN per mL against absorbance.

Linamarase activity was assayed by determining the HCN librated from linamarase as follows: 0.5 ml of enzyme solution in 0.2 M phosphate buffer (pH 6.5) contained in Eppendorf tubes was added to 0.5 ml of 1 mM buffered (same buffer) solution of linamarin (BDH, Poole, England). After 20 min of incubation at 32±2 °C, 2 mL of 2% KOH and 1 ml of picric acid:Na_2CO_3:H_2O (1 mL:5 g:200 mL) were added into the reaction mixture. The reaction was stopped by placing the tubes in iced water. The red colour that developed was read at 510 nm in a spectrophotometer. Under the above conditions, one unit of activity was defined as the amount of enzyme that released 1 μg HCN in 10 min under the assay condition.

Statistical analysis

The results are presented as the mean values of duplicate sampling. The significance of the tests was evaluated by analysis of variance (ANOVA). Significance of the test was accepted at ($p < 0.05$)

RESULTS AND DISCUSSION

Eight bacterial isolates which exhibited increased growth in the cyanide-containing medium were selected. The isolates designated RDS 6, RDS 12, RDS 13, RDS 20, RDS 26, RDS 28, RDS 31 and RDS 34 were selected for further studies because they grew and gave optical density readings of at least 0.6 in the cyanide containing medium (Table 1). The isolates were identified respectively as *Lactobacillus plantarum, Lactobacillus fermentum, Lactobacillus amylovorus, Lactobacillus cellobiosus, Leuconostoc mesenteroides, Pseudomonas stutzeri, Bacillus pumilus* and *Bacillus subtilis.*

Some nutritional factors relating to the production of linamarase enzyme by the isolates were investigated (Tables 2 and 3). There were no significant ($p > 0.05$) differences in the ability of standard media to cause more enzyme productivity by the isolates in comparison to locally formulated media. Under the conditions used, the enzyme activity that developed in the culture was higher in the presence of Tween 80 solution than in control experiments without Tween 80. Linamarase production by the isolates in media without Tween 80 showed the best enzyme activity of 2.67 U/mL produced with *Pseudomonas stutzeri* as the test organism. In comparison, best enzyme activity of 6.82 U/mL was obtained in the medium containing Tween 80 solution and *Lactobacillus fermentum* as the test bacterium. Incorporation of Tween 80 significantly ($p < 0.05$) increased the linamarase productivity of the isolates in all the media tested. This report agrees with the findings of Abalaka and Garba [16] who reported

increases in linamarase production in microbial culture media containing Tween 80 solution.

Table 1. Growth of the isolates in a medium containing 800 mg/L KCN solution

S/N	Isolate	OD at 600 nm
1	RDS 1	0.37
2	RDS 2	0.19
3	RDS 3	0.06
4	RDS 4	0.14
5	RDS 5	0.33
6	RDS 6	0.67
7	RDS 7	0.32
8	RDS 8	0.08
9	RDS 9	0.34
10	RDS 10	0.17
11	RDS 11	0.55
12	RDS 12	0.61
13	RDS 13	0.60
14	RDS 14	0.50
15	RDS 15	0.18
16	RDS 16	0.18
17	RDS 17	0.45
18	RDS 18	0.33
19	RDS 19	0.51
20	RDS 20	0.64
21	RDS 21	0.11
22	RDS 22	0.55
23	RDS 23	0.57
24	RDS 24	0.33
25	RDS 25	0.16
26	RDS 26	0.65
27	RDS 27	0.34
28	RDS 28	0.68
29	RDS 29	0.39
30	RDS 30	0.13
31	RDS 31	0.67
32	RDS 32	0.24
33	RDS 33	0.41
34	RDS 34	0.66
35	RDS 35	0.35
36	RDS 36	0.22
37	RDS 37	0.54
38	RDS 38	0.59
39	RDS 39	0.30
40	RDS 40	0.46

There are various reports on the simulative effects of surfactants in fermentation broth of microorganisms, thus resulting in many fold increases in the production and secretion of enzymes such as amylase [17,18], glucosetransferase [19], cellulase [20,21]; phytase [22], lignase [23], protease [24–26]. The surfactant may have improved cell wall permeability through disruption of lipid layer [27], thereby increasing the uptake of nutrient into the organisms and secretion of enzyme into the culture media.

This present work compared the rate of detoxification of cassava flour by microbial and endogenous cassava linamarases. The degradation of cyanogenic glucoside of cassava was studied in cassava flour pretreated to either prevent microbial enzyme activity or endogenous linamarase activity. The rate of disappearance of the cyanogenic glucosides as determined by the residual cyanide in the cassava flour was compared with the untreated control in which 1.98 mg HCN/10 g cassava flour remained unbroken after 80 h (Fig. 2). The residual cyanide in cassava flour samples treated with linamarase of *Lactobacillus plantarum* was undetectedin 30 h. In contrast, the residual cyanide in cassava flour samples treated with endogenous linamarase was 0.39 mg/10 g cassava flour after 80 h.

Two possible mechanisms of degradation of cassava linamarin are the introduction of microbial linamarase to cassava and cell wall – degrading enzymes that permit contact between the compartmentally separated linamarin and endogenous linamarase of cassava [28]. Gueguen *et al.* [29] reported that cassava is detoxified during processing by the endogenous linamarase present in the enlarged cassava root during grating of the root but the quantity of enzyme released is not sufficient to break down the glucoside present in the root completely. The authors suggested that the endogenous linamarase of the root could be supplemented from a microbial source exogenous to the roots to ensure exogenous to the roots to ensure a greater breakdown of the linamarin. Mkpong *et al.* [30] and Ikediobi and Onyike [31] reported that the endogenous linamarase content could not permit the complete breakdown of linamarin. Ikediobi and Onyike [31] and Okafor and Ejiofor [13] demonstrated that it is possible to reduce the cassava toxicity by the addition of an exogenous linamarase during the fermentation. Petruccioli *et al.* [32], reported in 1999 that addition of *Mucor circinelloides* crude linamarase during cassava tuber fermentation shortened and enhanced the detoxification process leading to a complete hydrolysis of cassava cyanogenic glucoside. In contrast, Giraud *et al.* [33] observed that inoculation of cassava pulp with a strain of lactic acid bacteria possessing a strong linamarase activity did not appear to contribute to cassava detoxification. The authors concluded that the amount of cassava indigenous linamarase released during the grating stage was sufficient to permit complete and rapid hydrolysis of cassava linamarin. This report agrees with Vasconcelos *et al.* [34] who stated that 95% of initial linamarin was hydrolyzed 3 h after grating the

Table 2. Production of linamarase by the isolates in different media without Tween 80 solution – total activity units in 40% $(NH_4)_2SO_4$ fraction (mean ± SD)

Isolate	Banana broth	Orange broth	Tomato juice broth	MRS broth	Czapek dox broth	Nutrient broth
Lactobacillus plantarum	0.26±0.028	1.33±0.41	2.18±0.099	2.11±0.180	0.12±0.042	0.17±0.042
Lactobacillus fermentum	0.29±0.048	0.76±0.021	2.08±0.056	2.13±0.014	0.43±0.021	0.16±0.014
Lactobacillus amylovorus	0.21±0.028	0.53±0.070	1.58±0.184	1.88±0.550	0.24±0.198	0.09±0.057
Lactobacillus cellobiosus	0.39±0.127	0.46±0.090	1.40±0.400	1.31±0.020	0.26±0.093	0.18±0.028
Leuconostoc mesenteroides	0.20±0.127	0.88±0.113	2.06±0.350	1.46±0.085	0.35±0.078	0.30±0.100
Pseudomonas stutzeri	2.67±0.099	1.77±0.042	0.12±0.042	0.15±0.085	0.48±0.099	2.36±0.240
Bacilus pumilus	2.46±0.099	1.61±0.040	0.08±0.071	0.19±0.170	0.23±0.042	2.55±0.35
Bacilus subtilis	2.18±0.098	2.40±0.032	0.15±0.040	0.28±0.099	2.56±0.052	0.42±0.042

Table 3. Production of linamarase by the isolates in different media with Tween 80 solution – total activity units in 40% $(NH_4)_2SO_4$ fraction (mean ± SD)

Isolate	Banana broth	Orange broth	Tomato juice broth	MRS broth	Czapek dox broth	Nutrient broth
Lactobacillus plantarum	3.43±0.042	2.53±0.100	5.96±0.230	5.62±0.420	1.94±0.570	2.22±0.230
Lactobacillus fermentum	2.09±0.049	3.66±0.44	6.82±0.82	4.88±0.049	4.16±0.035	2.67±0.047
Lactobacillus amylovorus	2.60±0.070	2.86±0.300	5.65±0.040	4.80±0.680	2.46±0.070	2.85±0.510
Lactobacillus cellobiosus	4.92±0.140	4.34±0.070	4.21±0.160	5.28±0.170	3.13±0.180	3.58±0.040
Leuconostoc mesenteroides	3.44±0.180	3.57±0.040	5.18±0.240	4.65±0.210	2.89±0.170	2.07±0.100
Pseudomonas stutzeri	4.29±0.610	4.25±0.042	3.37±0.060	2.91±0.140	4.61±0.110	5.16±0.240
Bacilus pumilus	6.61±0.590	5.90±0.300	2.84±0.310	3.40±0.170	4.93±0.042	6.09±0.090
Bacilus subtilis	5.78±0.170	6.17±0.400	2.63±0.200	3.88±0.130	5.11±0.230	2.40±0.030

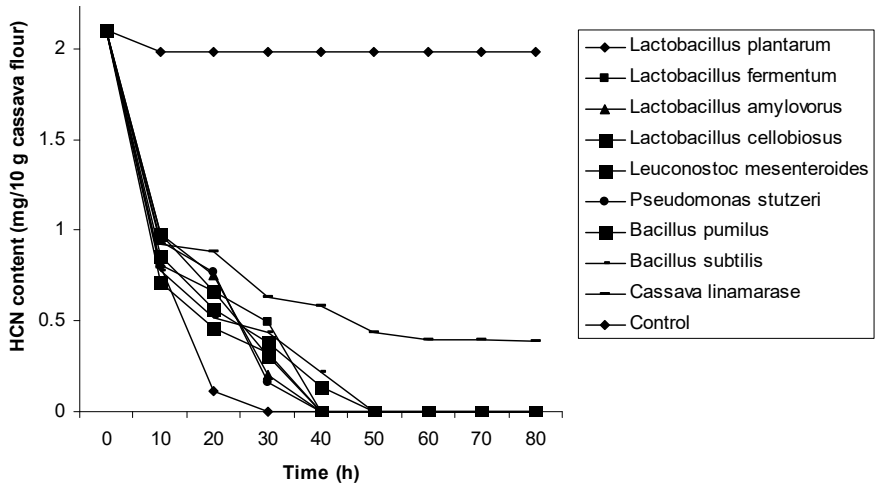

Figure 2. Comparison of the rate of detoxification of cassava flour by microbial and endogenous cassava linamarase enzymes.

roots. Maduagwu [35] observed that the degradation of cyanogenic glucoside in mashed cassava roots during fermentation was essentially effected by endogenous linamarase and that the role of microbial linamarase activity was complementary.

CONCLUSION

Eight linamarase-producing bacteria were selected based on their high growth in media containing 800 mg/L potassium cyanide solution. The isolates showed very high growth in the growth medium as demonstrated by increase in their optical density readings to at least 0.6. The isolates were identified as *Lactobacillus plantarum, Lactobacillus fermentum, Lactobacillus amylovorus, Lactobacillus cellobiosus, Leuconostoc mesenteroides, Pseudomonas stutzeri, Bacillus pumilus* and *Bacillus subtilis.* All the isolates were grown in media containing Tween 80 solution and in control media without the surfactant. Best enzyme activity of 6.82 U/mL was obtained in the medium containing Tween 80 solution and *Lactobacillus fermentum* as the test bacterium. Linamarase production by the isolates in media without Tween 80 was much lower

than in media with the surfactant. Residual cyanide in cassava flour samples treated with linamarase of *Lactobacillus plantarum* was undetected in 30 h; while in contrast, the residual cyanide in cassava flour samples treated with endogenous cassava linamarase was 0.39 mg/10 g cassava flour after 80 h. Residual cyanide in the untreated control sample was 1.98 mg HCN/10 g cassava flour after 80 h.

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IZVOD

PROIZVODNJA LINAMARAZE POMOĆU MIKROORGANIZAMA I POREĐENJE BRZINE RAZGRADNJE CIJANIDA KASAVE MIKROBNIM LINAMARAZAMA I LINAMARAZAMA KASAVE

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(Naučni rad)

U radu je proučena mikrobna proizvodnja linamaraze i uticaj sastava hranljive podloge na proizvodnju enzima. Izolovano je ukupno osam bakterija producenata linamaraze iz krtola kasave i tla. Izbor izolata je izvršen na osnovu kriterijuma brzog rasta u medijumu koji sadrži 800 mg/L kalijum-cijanida. Osam izolata, koji su pokazali vrlo brz rast u hranljivoj podlozi sa cijanidom detektovan rastom optičke gustine do 0,6, izabrani su za dalje istraživanje. Ovi izolati su identifikovani kao: *Lactobacillus plantarum, Lactobacillus fermentum, Lactobacillus amylovorus, Lactobacillus cellobiosus, Leuconostoc mesenteroides, Pseudomonas stutzeri, Bacillus pumilus* i *Bacillus subtilis*. Svi izolati su gajeni u medijumu koji sadrži Tween 80 i u kontrolnom medijumu bez ovog surfaktanta. Najviša enzimska aktivnost od 6,82 U/ml dobijena je u medijumu koji sadrži Tween 80 i sa *Lactobacillus fermentum* kao testiranim mikroorganizmom. Mikrobnom proizvodnjom linamaraze u medijumu bez Tween 80 postignuta je manja enzimska aktivnost. Sposobnost endogenih enzima kasave i mikrobnih enzima da hidrolizuju cijanide brašna kasave je testirana na uzorcima brašna pretretiranim radi uklanjanja enzima prisutnih u brašnu. Cijanidi u brašnu tretiranom linamarazom iz *Lactobacillus plantarum* nakon 30 h nisu bili detektovani, dok je u uzorcima tretiranim endogenim linamarazama kasave nakon 80 h detektovano 0,39 mg/10 g kasavinog brašna. Koncentracija cijanida u kontrolnom uzorku, bez enzima, nakon 80 h je bila 1,98 mg HCN/10 g brašna. Rezultati ovog istraživanja pokazuju da primena mikrobnih linamaraza donosi unapređenje u razgradnji cijanida kasave u odnosu na razgradnju zasnovanu samo na endogenim linamarazama. Inokuliranje fermentišućih krtola kasave mikrobnim izolatima selektovanim u ovom radu će omogućiti bolju kontrolu procesa fermentacije kasave i može da dovede do proizvodnje standardizovanih i netoksičnih prehrambenih proizvoda od kasave.

Ključne reči: Linamaraza • Smanjenje koncentracije cijanida kasave • Mikroorganizmi • Surfactant