Egyptian Academic Journal of Biological Sciences is the official English language journal of the Egyptian Society for Biological Sciences, Department of Entomology, Faculty of Sciences Ain Shams University.

Microbiology journal is one of the series issued twice by the Egyptian Academic Journal of Biological Sciences, and is devoted to publication of original papers related to the research across the whole spectrum of the subject. These including bacteriology, virology, mycology and parasitology. In addition, the journal promotes research on the impact of living organisms on their environment with emphasis on subjects such a resource, depletion, pollution, biodiversity, ecosystem…..etc

www.eajbs.eg.net

Citation: *Egypt. Acad. J. Biolog. Sci. (G. Microbiolog) Vol.9 (1)pp. 73- 83 (2017)*

Egypt. Acad. J. Biolog. Sci., 9(1): 73-83 (2017)

Egyptian Academic Journal of Biological Sciences
G. Microbiology

ISSN: 2090-0872
www.eajbs.eg.net

In vitro Degradation of Extracted Cassava Linamarin by *Bacillus* Species Isolated from cassava wastewater

Ogunyemi, A.K[1], Samuel, T.A[2], Buraimoh, O.M[1], Amund, O.O[1], Ilori, M.O[1], Alagbada, B.C[3], Olumuyiwa, E.O[4], Odetunde, S. K[5] and Akinrodoye, F[2]

1- Department of Microbiology, University of Lagos, Akoka, Lagos-Nigeria
2- Department of Biochemistry, College of Medicine, University of Lagos, Idi-Araba, Lagos-Nigeria
3- Department of Chemical Sciences, Yaba College of Technology, Yaba, Lagos-Nigeria
4- Department of Biological Sciences, College of Natural and Applied Sciences, Bells University of Technology, Ota, Ogun- Nigeria.-
5- Department of Science Laboratory Technology, Microbiology Unit, Lagos State Polytechnic, Ikorodu, Lagos-Nigeria
*Correspondence Author: waleogunyemi2002 @yahoo.com

ARTICLE INFO

Article History
Received:25/4/2017
Accepted:28/5/2017

Keywords:
Bacillus pumilus
Biodegradation
Intermediates
Linamarase
Bacterial species
Cassava wastewater

ABSTRACT

Linamarin is the most abundant cyanoglucoside present in cassava cells and may generate the equivalent amount of hydrocyanic acid. This study was aimed to assess degradative capacities of *Bacillus pumilus* strain WOB3 and WOB7on linamarin. The test organisms for linamarase activity were identified on the basis of phenotype, biochemical properties and 16S rDNA gene sequencing as: *Bacillus pumilus* strain WOB3 KX774195 and *Bacillus pumilus* strain WOB7 KX774196. Growth studies showed that the strains grew in all the substrates tested. The doubling times of *Bacillus pumilus* strain WOB3 and *Bacillus pumilus* strain WOB7 were 8.25 d and 7.53 d on cassava effluent, 6.30 d and 5.78 d on supplemented cassava effluent, 8.66 d and 9.90 d on waste leachate and 6.30 d and 9.24 d on supplemented waste leachate respectively; with specific growth rates of 0.084 d^{-1} and 0.092 d^{-1} on cassava effluent, 0.11 d^{-1} and 0.12 d^{-1} on supplemented cassava effluent, 0.080 d^{-1} and 0.070 d^{-1} on waste leachate and 0.11 d^{-1} and 0.075 d^{-1} on supplemented waste leachate respectively. High-performance liquid chromatographic analysis studies revealed that linamarin degradation by the strains followed a linamarase pathway involving CO_2 and HCN as metabolic intermediates. Based on HPLC analysis, linamarin residual concentration at day 12 by the strains WOB3 and WOB7 was 26.73 mgL^{-1}(19.79 %) and 29.79 mgL^{-1} (21.92 %). These novel features make the bacteria suitable candidates for in-situ application on sites contaminated with cassava processing wastes.

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) roots and leaves contain high concentrations of linamarin (alphahydroxyisobutyronitrile- beta-*D*-glucopyranoside) and lotaustralin (methyl-linamarin) (Vasconcellos *et al*., 2009). Linamarin is the most abundant cyanoglucoside present in cassava cells (Conn, 1969) and may generate the equivalent to 0.2-100 mg of HCN per 100 g of fresh cassava following cellular lysis (Bradbury *et al*., 1991).

Microorganisms can grow on substrates containing cyanides by anaerobic metabolism, or by using an aerobic respiration chain as an alternative pathway (Cereda *et al*., 1981). In both pathways, HCN is eliminated from the substrate, and converted into a non-toxic product (Jensen and Abdel-Ghafar, 1979).

The current global concern with environmental problems has made the biological detoxification of cyanide an attractive alternative to chemical detoxification procedures (Meyers *et al*., 1991). Biological detoxification methods may be both cheaper and more environmentally acceptable than chemical methods. Most attempts to develop biological processes for the detoxification of cyanide-containing effluents from industry have concentrated on cyanide degrading fungi (Knowles and Bunch, 1986). This enzymatic cyanide-removing property can be exploited for the detoxification of cyanide-rich cassava wastewater and industrial residues. These residues currently cause serious environmental problems in many cassava flour producing plants in Nigeria, one of the largest producers worldwide, and in many African, Latin American and Asian countries (Romero *et al*., 2002). However, none has achieved complete detoxification (Blanshard *et al*., 1994) and in spite of the availability of several processing techniques, cyanide exposure from cassava diets prevails (Ernesto *et al*., 2000). Cassava roots can be industrially applied for obtaining starch and flour. However, cassava industries generate some undesirable sub-products, such as solid residues and a liquid effluent named manipueira, which may represent a major disposal problem due to the high organic charge and toxic potential, resulting from the presence of cyanoglucosides (Cereda, 2001). Cyanoglucosides are secondary metabolites produced by several plant species (Conn, 1994) used in animal and human diets, such as apple, bamboo shoot, cassava, cherry, lima bean, maize, oat, peach, papaya, sorghum and wheat (Pitcher *et al*., 1989). These compounds are dispersed throughout the plant organs, mostly in non-edible parts (Jones, 1978), but may become concentrated in edible roots and leaves, as in the case of cassava. Microorganisms can grow on substrates containing cyanides by anaerobic metabolism, or by using an aerobic respiration chain as an alternative pathway (Cereda *et al*., 1981). In both pathways, HCN is eliminated from the substrate, and converted into a non-toxic product (Jensen and Abdel-Ghafar, 1979). This enzymatic cyanide-removing property can be exploited for the detoxification of cyanide-rich cassava wastewater and industrial residues. These residues currently cause serious environmental problems in many cassava flour producing plants in Brazil, the largest producer worldwide, and in many African, Latin American and Asian countries (Romero *et al*., 2002), where cassava products are an important input for human diet. Manipueira is rich in potassium, nitrogen, magnesium, phosphorous, calcium, sulfur and iron, presenting a great potential as an agronomic fertilizer. It contains cyanoglucosides, which explains the application as nematicide and insecticide (Palmisano *et al*., 2000). The study intent was to evaluate the potentials of two bacterial strains in bioremediative clean-up of the polluted environment and elucidate the degradation pathway of linamarin contained in the waste products.

MATERIALS AND METHODS

Microorganisms and inoculum development

Linamarin-utilizing bacterial strains were isolated and identified as previously described by Ahaotu *et al*. (2013). They were identified on the basis of morphological and biochemical characteristics and 16S rRNA gene sequencing. The bacterial isolates are *Bacillus pumulis* strain WOB3 KX774195 and *Bacillus pumilus* strain WOB7 KX774196. In previous laboratory studies, these strains have demonstrated substantial capabilities to utilize linamarin for growth.

They also showed varied abilities to utilize other natural substrates such as solid waste leachate. The isolates were resuscitated using nutrient agar and pre-enrichment was carried using Luria-Bertani broth.

Extraction of Linamarin from cassava tuber

Extracts were obtained from the same cultivars of cassava growing in the cassava plant farm at Lagos State Polytechnic, Ikorodu, Lagos. Preliminary extractions were carried out using cassava roots in phosphoric acid organic solvents as described by King and Bradbury (1995), but the final aqueous method used was as follows: A 800 g sample of very young cassava root was cut up with stainless knife and scissors and immediately ground in a glass pestle and mortar with 80 mL of 0.1M phosphoric acid. A further 80 mL of 0.1 M phosphoric acid were added with further grinding and the pasty solution was sieved through a cloth which was squeezed. The pink-coloured, cloudy solution was then centrifuged and the clear, supernatant liquid removed with a Pasteur pipette. This solution, which also contained linamarase (inactivated in the 0.1M phosphoric acid) and linamarin, was stored frozen in a deep freezer cabinet at 20 °C.

Bacterial growth studies on cassava effluent and waste leachate

Growth study of the microbial strains was performed under aerobic batch conditions. Turbidity method was used for the growth study of the strains. Organisms were grown in Erlenmeyer flasks (250 mL) containing cassava effluent (20 $\%^{v}/_{v}$; 100 mL; pH 7.2). The medium containing linamarin as substrate as sole carbon source was autoclaved at 121 °C and was inoculated with 1.0 mL of inoculum. Apart from cassava effluent, the bacterial isolates also grew on waste leachate. Growth was evaluated at intervals (2 days) by the intensity of turbidity ($O.D_{600\ nm}$) in cassava effluent medium. A positive control flask contained the inoculum but without the substrate while the negative control contained the substrate but without the inoculum. The growth cultures were set up in duplicates. The culture fluid (5.0 mL) from the two sets of flasks was harvested every 2 days for determination of optical density and pH change.

Substrate Utilization by test Organisms

Similarly, for linamarin degradation studies, organisms were grown in Erlenmeyer flasks (500 mL) containing minimal salts medium (250 mL; pH 7.2) with extracted linamarin (0.5 %w/v) and fortified with 1.0 mL of trace elements solution (Obayori *et al*., 2009). This was autoclaved, allowed to cool before inoculation with 2.0 mL of the inoculum and incubation was done on a rotary shaker (28 ^{o}C, 150 rpm) for 12 days. The culture fluid (25.0 mL) from the two sets of flasks was harvested every 2 days for high-performance liquid chromatographic analysis to determine residual linamarin and detect other possible metabolites in the culture fluids.

Analysis of growth kinetic data

Mean generation times (T_d) and the specific growth rates (μ) of the bacterial strains on linamarin as carbon source were computed with non-linear regression of growth curves using Graph pad software prism version 6. Briefly, an "XY "data table was created. Time (days) was entered into "X", and response (cell optical density and pH) into "Y"- axis respectively. Analysis was carried out by clicking "analyse", choosing the non-linear regression panel of exponential equations and exponential growth respectively. The parameter Y0 (value at time zero) was constrained to a constant value by going to the constrain tab of the non-linear regression dialog, setting the drop down next to Y0 to "constant equal to". The values were entered. The model Y=Y0*Expo (k*X) was used; where Y0 is the Y value when X (time) is zero, K is the rate constant, expressed in reciprocal of the X-axis time units. Doubling time was computed as ln 2/K (Buraimoh *et al*., 2017).

High-Performance Liquid Chromatographic Analysis (HPLC)

Determination of the residual linamarin content in the media during the period of incubation was carried out using high-

performance liquid chromatography (Schimadzu Kyoto, Japan) with a flame ionization detector. Instrument operating conditions were as follows: an μbandapak C-18 glass column pack with internal diameter of 5.0 μm and length of 250 mm packed with porapak N, 60/100, a column temperature of 200 ^{o}C, an injector temperature of 28 ^{o}C, a detector temperature of 280 ^{o}C, N_2 carrier gas and H_2 at a flow rate of 1.5 mL/min and temperature/ramping rate of 5 ^{o}C/min. A standard profile was first obtained by injecting 5.0 mL of the linamarin into the HPLC and a chromatogram was generated to serve as a calibration window with which the test sample was analyzed, after which 20.0 mL of the growth culture was extracted with 20 mL acetonitrile, and then concentrated to 5.0 mL (each test sample) from which 1 μL was injected into the HPLC column and an equivalent chromatogram was generated to detect the metabolites of biodegradation. The peak areas of the standard and test sample chromatograms were compared and were used to calculate the concentration of the sample, given by the formula:

$$\text{Concentration of Linamarin} = \frac{\text{Total peak of sample} \times \text{Concentration of standard}}{\text{Peak area of standard}}$$

RESULTS

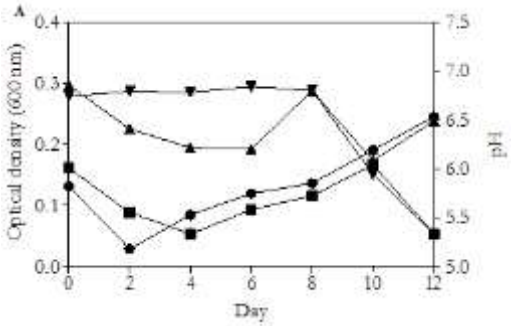
Growth of *Bacillus pumilus* (strains WOB3 and WOB7) on cassava effluent and waste leachate

Table 1 shows doubling times and specific growth rates of test organisms on cassava effluent (supplemented with or without minimal salts). While Fig. 1 indicates the growth profiles of the test organisms on cassava effluent only and supplemented cassava effluent. When grown on cassava effluent, strain WOB7 had higher specific growth rate of 0.092 d^{-1} with doubling time of 7.53 d than strain WOB3 (0.084 d^{-1} , 8.25 d). An increase in specific growth rates by strains WOB3 and WOB7 was observed when grown on cassava effluent supplemented with minimal salts and specific growth rates of 0.11 and 0.12 d^{-1} were recorded respectively. Apart from using linamarin in cassava effluent, the test organisms also grew on waste leachate only and when supplemented with minimal salts.

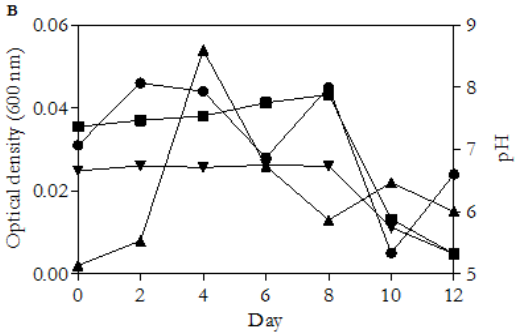
Table 1: Growth potentials of linamarin-utilizing bacteria grown on cassava effluent and waste leachate (supplemented with or without minimal salts)

Isolates	Substrates							
	C		C+M		W		W+M	
	μ (d^{-1})	T_d (d)	μ (d^{-1})	T_d (d)	μ (d^{-1})	T_d (d)	μ (d^{-1})	T_d (d)
WOB3	0.084	8.25	0.11	6.30	0.080	8.66	0.11	6.30
WOB7	0.092	7.53	0.12	5.78	0.070	9.90	0.075	9.24

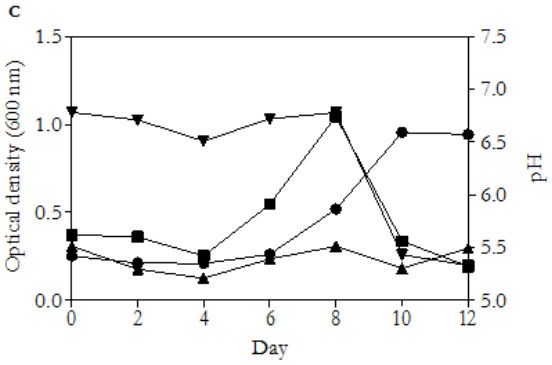
C=Cassava effluent only; C+M=Cassava effluent plus minimal salts, W=Waste leachate only, W+M=Waste leachate plus minimal salts, μ-Specific growth rate, T_d-Doubling time, d-Day



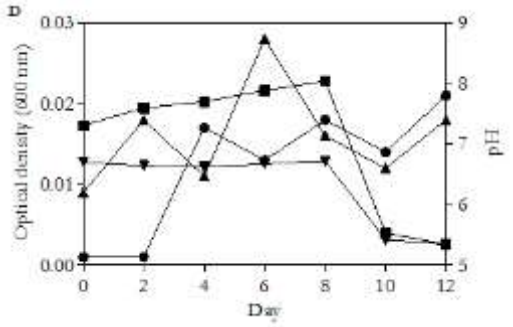
A-Growth of *Bacillus pumilus* strain WOB3 on cassava effluent only (without minimal salts) and supplemented cassava effluent (with minimal salts).



B-Growth of *Bacillus pumilus* strain WOB3 on waste leachate only (without minimal salts) and supplemented waste leachate (with minimal salts).



C- Growth of *Bacillus pumilus* strain WOB7 on cassava effluent only (without minimal salts) and supplemented cassava effluent (with minimal salts)



D-Growth of *Bacillus pumilus* strain WOB7 on waste leachate only (without minimal salts) and supplemented waste leachate (with minimal salts)

Fig. 1: Growth profiles and pH changes in the culture fluids of *Bacillus pumilus* strain WOB3 and *Bacillus pumilus* strain WOB7

●-Optical density (substrate only); ▲- Optical density (supplemented substrate); ■ -pH changes (substrate only); ▼ -pH changes (supplemented substrate)

The change in pH

Generally, there were dramatic changes in pH of the culture fluids which tends towards acidic direction from neutral as shown in Table 2. Continuous monitoring of pH revealed a drop in pH (7.2–5.33) of the cassava effluent medium with or without minimal salts, in both cases respectively, when inoculated with WOB3 strain. There is little or no difference in the pH of the culture medium using the same strain on leachate with or without salts (7.2-5.32). When *Bacillus pumilus* strain WOB7 was grown on cassava effluent medium with or without minimal salts, the pH of the culture medium was 7.2–5.32 and 7.2-5.33, respectively. Similarly, in leachate medium with or without minimal salts, the pH of the culture medium was 7.2–5.34, in both cases respectively (Fig. 1).

Table 2: pH changes in the culture fluids having test organisms grown on cassava effluent and waste leachate (supplemented with or without minimal salts)

Days	Organisms							
	WOB3				WOB7			
	C	C+M	L	W+M	C	C+M	W	W+M
0	6.01	6.74	7.37	6.66	5.62	6.78	7.31	6.70
2	5.55	6.79	7.47	6.74	5.60	6.71	7.59	6.64
4	5.33	6.78	7.54	6.71	5.42	6.51	7.70	6.62
6	5.58	6.84	7.75	6.75	5.91	6.72	7.88	6.67
8	5.72	6.80	7.88	6.73	6.74	6.78	8.04	6.72
10	6.04	5.94	5.88	5.75	5.56	5.43	5.53	5.42
12	5.33	5.33	5.32	5.32	5.32	5.33	5.34	5.34

C=Cassava effluent only; C+M=Cassava effluent plus minimal salts; W=Waste leachate only; W+M=Waste leachate plus minimal salts

Extraction of Linamarin from cassava tuber

The amount of linamarin in the pink solution produced from crushed root was assayed using 25.0 mL aliquots and a calculation gave the amount of linamarin present in the root. The linamarin content of very young root of cultivars was 137.18 mg/L. The linamarin solution from of this cultivar was concentrated enough to be used for biodegradative study.

HPLC Analysis of the Products of Degradation of Linamarin by *Bacillus pumilus*

The degradation of linamarin and formation of other possible metabolites were analyzed by high-performance liquid chromatography (HPLC). The strains WOB3 and WOB7 were able to degrade 80.21 and 78.08 % linamarin, after 12 days respectively. The results of HPLC analysis showed that strain WOB3 had degraded linamarin from 135.07 to 26.73 mg/L while 6.01-8.71 mg/L of hydrocyanic acid (HCN) and 0.70-12.25 %v carbon dioxide (CO_2) had accumulated as possible metabolites whereas strain WOB7 degraded of 135.90 to 29.79 mg/L with formation of 2.77-8.73 mg/L of hydrocyanic acid (HCN) and 3.95-9.32 %v carbon dioxide (CO_2) (Fig. 2). There was a dramatic decrease in the concentration of linamarin as clearly indicated in the reduction of the peaks after 12 days of incubation for the two bacterial species (Fig. 3). The retention times of the authentic compounds and the metabolites detected are given in Table 3. In the control experiment, the production of hydrocyanic acid and carbon dioxide was not observed in culture flasks devoid of linamarin as well as in uninoculated flasks containing linamarin. This confirmed the utilization of linamarin from the formation of these metabolites as a consequence of hydrolysis of linamarin by *Bacillus pumilus* strain WOB3 and *Bacillus pumilus* strain WOB7. On the whole, the utilization of linamarin by these strains indicates that these organisms are promising candidates for the removal of cyanogenic

wastes from contaminated wastewater. Also, the results of HPLC analysis showed that the two bacterial strains, *Bacillus pumilus* strain WOB3 and *Bacillus pumilus* strain WOB7 converted the linamarin into hydrocyanic acid, carbon dioxide and acetone (Fig. 4).

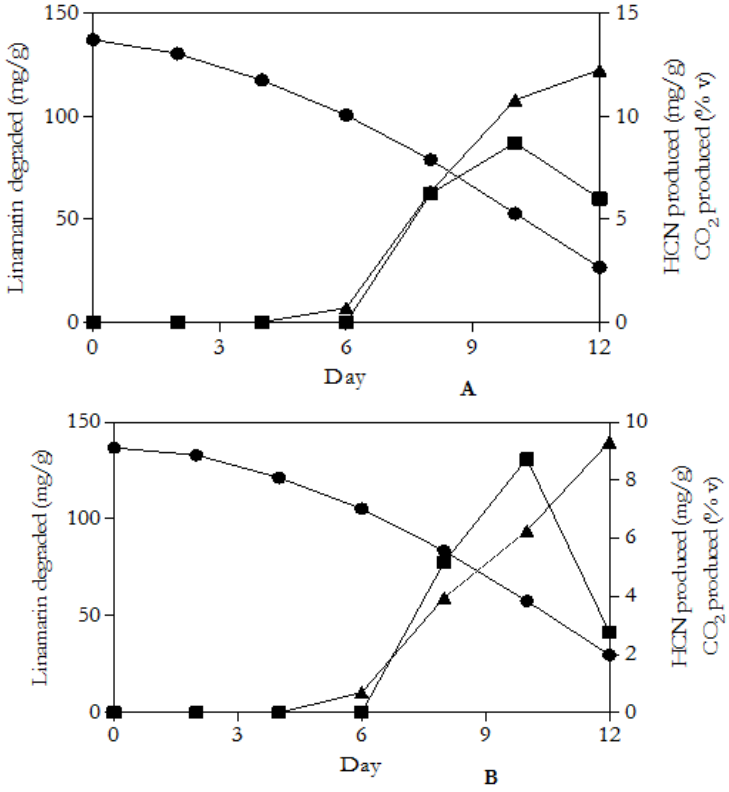
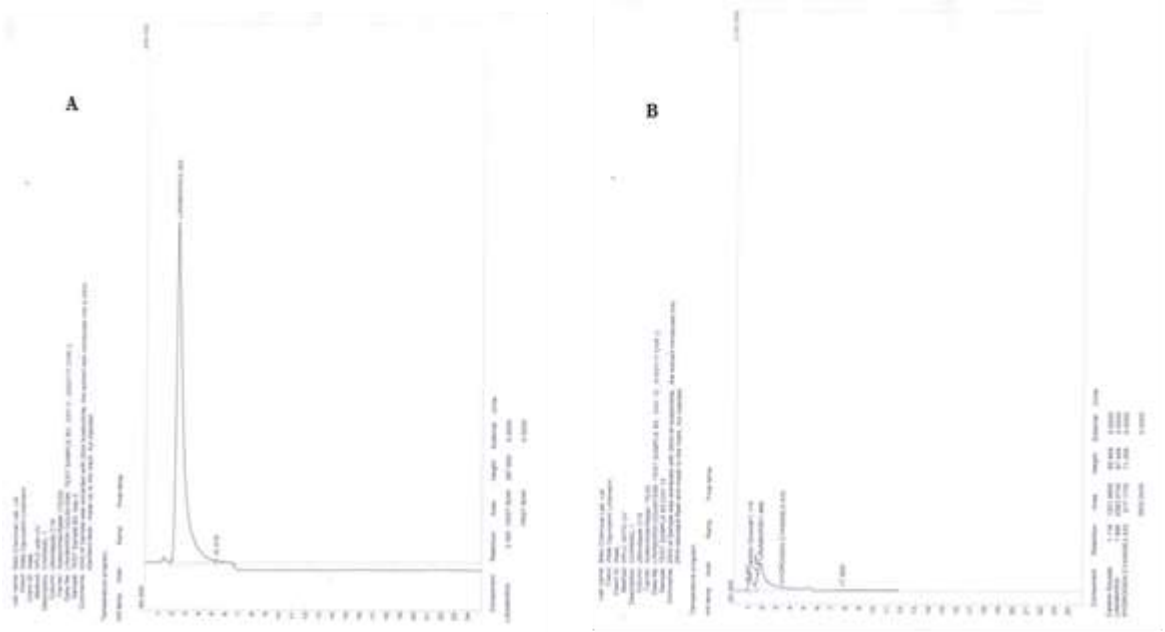


Fig. 2: Degradation of Linamarin and other possible product formation by *Bacillus pumilus* strains WOB3 (A) and *Bacillus pumilus* strain WOB7 (B); ●-linamarin; ■ - HCN-Hydrogen cyanide; ▲ -CO_2-Carbon dioxide;



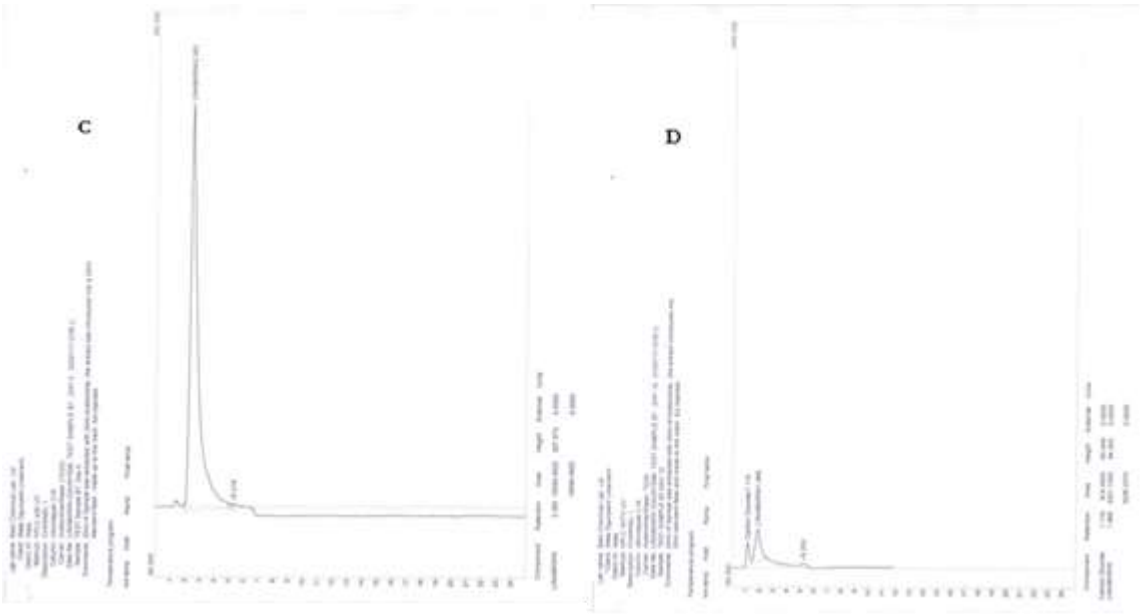


Fig. 3: HPLC analysis of linamarin degradation products by *Bacillus pumilus strain* WOB3 (A-day 0 and B-day 12) and *Bacillus pumilus* strain WOB7 (C-day 0 and D-day 12) after different incubation times.

Table 3: High-performance liquid chromatographic analysis of authentic standard compounds as compared to peaks of metabolites detected in culture filtrates

S.I. NO.	Compounds	WOB3 Retention times (min) of		WOB7 Retention times (min) of	
		Authentic standards	Metabolites	Authentic standards	Metabolites
1	Linamarin	2.383	1.866	2.383	1.866
2	Hydrocyanic acid (HCN)	3.566	3.433	3.766	3.533
3	Carbon dioxide (CO_2)	1.383	1.116	1.200	1.116

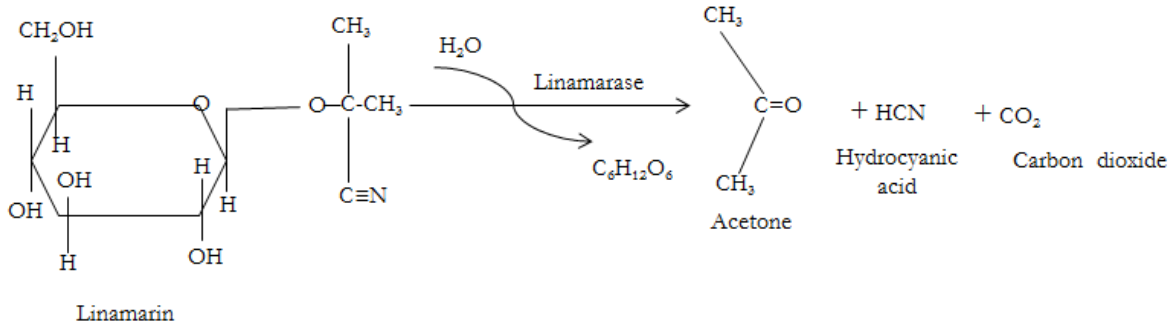


Fig. 4: Proposed catabolic routes of Linamarin degradation by *Bacillus pumilus* strain WOB3 and *Bacillus pumilus* strain WOB7

DISCUSSION

Some studies on cyanoglucoside degradation by *Candida tropicalis* and *Candida utilis*, exist in literature but these studies did not report the degradation rates (Legras *et al*., 1990; Fagbemi and Ijah, 2006; Vasconcellos *et al*., 2009). To the best of our knowledge, this was first time where other products of linamarin degradation were detected using high performance liquid chromatography (HPLC). Essers *et al*. (1995) reported that several fungi and bacteria were isolated from Ugandan domestic fermented cassava, which released HCN from linamarin in defined growth media. They further found out that in 72 h, a *Bacillus* sp. decreased the linamarin to 1 % of initial concentrations while *Mucor racemosus*, *Rhizopus oryzae* and *R. stolonifer* decreased it to 7 and 30 %,

respectively whereas *Neurospora sitophila* and *Geotrichum candidum* hardly degraded the linamarin. Ugwuanyi *et al*. (2007) described *Bacillus* species including *Bacillus coagulans*, *Bacillus licheniformis* and *Bacillus stearothermophilus*, able to degrade linamarin. The use of HPLC for the quantification of the microbial linamarin degradation and other metabolites (HCN and CO_2) was an innovation introduced in the present work. The results obtained herein revealed the potentials of two *Bacillus pumilus* strains with high capacities for biodegrading the cyanoglucoside linamarin. In this study, at the end of incubation period of day 12, the strainWOB3 was able to degrade linamarin from an initial concentration of 137.18 to final concentration of 26.73 mg/L (80.52 %) while strain WOB7 was able to degrade linamarin from concentration of 135.90 mg/L to final concentration of 29.79 mg/L (78.08 %). In similar study by Vasconcellos *et al*. (2009), the test organism was able to degrade linamarin from an initial concentration of 7.47 mg/mL to final concentration of 2.16 mg/mL (71 %) after seven days. Murugan *et al*. (2012) reported the utilization of cyanogenic glycoside by *Bacillus subtilis* KM05 which went through assimilatory degradation with the release of hydrogen cyanide and ammonia. Also, the formation of HCN and release of ammonia as an end product evidenced the assimilatory degradation of linamarin. Kobawila *et al*. (2005) reported reduction of the cyanide content during fermentation of cassava roots and leaves to produce bikedi and ntoba mbodi, two food products from Congo. The cyanide content decreases during the fermentation of cassava roots and leaves by more than 70 % through the activities of the bacterial produced linamarase, allowing the hydrolysis of cyanogenic glucosides. Certain lactic bacteria present in the environment of fermentation are resistant to the strong cyanide concentrations of between 200 and 800 ppm. Vasconcelos *et al*. (1990) observed that the degradation of cyanogenic compounds during the fermentation of cassava, leads to the accumulation of free cyanide, which can reach 200 ppm in the fermenting medium. The linamarase produced by the cassava lactic acid bacteria, notably *Leuconostoc mesenteroides* and *Lactococcus lactis*, and the endogenous linamarase contribute to the process of detoxification. Besides, hydrolysis of cyanogenic glucosides (Louembe *et al*., 1997; Kobawila *et al*., 2003; Vaconcelos *et al*., 1990; Okafor and Ejiofor, 1986) takes place in acid environment (pH 3.8) during lactic fermentation of cassava roots as well as in basic environment (pH 8.5) during alkaline fermentation of the cassava leaves. Forty *Bacillus* spp. including *Bacillus subtilis* (20), *Bacillus licheniformis* (11), *Bacillus sonorensis* (7), *Bacillus cereus* (2) isolated from acid fermented primary starters, were screened by Abban *et al*. (2012) for their ability to hydrolyze and to grow on linamarin at pH levels below 5.0. Abban *et al*. (2012) found that *B. licheniformis* and *B. sonorensis* isolates grew and degraded cyanogens at pH 5.0 in a HCl environment, while two *B. cereus* isolates used in the study showed no breakdown reaction under all conditions tested. The decrease of pH during the fermentation of cassava roots results from the production of organic acids by lactic acid bacteria, which constitute the dominant microflora (Malonga *et al*., 1993; Malonga *et al*., 1996). The alkaline pH during the fermentation of cassava leaves could be due to amines produced by *Bacillus* (Louembe *et al*., 2003). In this study, similar findings were obtained in the culture fluid during the incubation period. Certain strains of *Bacillus*, notably *Bacillus pumilus*, have the capacity to use cyanhydric acid for their nutrition (Knowles, 1976; Skowronski *et al*., 1969). They can thus contribute to the reduction of the cyanide content in the medium of fermentation. The alkaline pH would facilitate reduction of the cyanogenic glucosides content because cyanohydrin acetone, produced by the hydrolysis of linamarin, is cleaved spontaneously when pH is above 5.0 or by the action of hydroxynitril

lyase to give acetone and cyanhydric acid (Nartley, 1968; Conn, 1969; Cooke *et al*., 1978; Formunyam *et al*., 1985).

CONCLUSION

The results obtained in this study revealed the high abilities of two *Bacillus pumilus* strains for biodegrading the cyanoglucoside linamarin. The use of such microorganisms in plants´ treatment for cassava industries, which are widely distributed in Nigeria, may offer a great potential for detoxification of cassava wastewaters for future application in fertirrigation.

ACKNOWLEDGEMENTS

The authors would like to thank to Mr Joe of Bato Laboratories at Coker Aguda, Lagos, Nigeria for the help with the HPLC analysis. We are grateful to Prof. Michael Benedick of Texas A&M University, TX 77843, USA and Mr. Olayinka Onifade of University of Lagos, Biochemistry Department, College of Medicine, University of Lagos, Lagos, Nigeria for technical guidance and support.

REFERENCES

Abban, S, Leon Brimer L, Abdelgadir WS, Jakobsen M., Thorsen L. (2012). Screening for *Bacillus subtilis* group isolates that degrade cyanogens at pH 4.5–5.0. Int J Food Microbiol., 161: 31-35.

Ahaotu I, Ogueke CC, Owuamanam CI, Ahaotu NN, Nwosu JN. (2011). Fermentation of undewatered cassava pulp by linamarase producing microorganisms: effect on nutritional composition and residual cyanide. Am J Food Nutr., 3(1):1-8.

Blanshard AFJ, Dahniya MT, Poulter NH, Taylor AJ. (1994). Fermentation of cassava into Foofoo: Effect of time and temperature on processing and storage quality. J Sci Food Agric., 66(4): 485–492.

Buraimoh OM, Ilori MO, Amund OO, Isanbor C, Michel Jr. FC. (2017). The degradation of coniferyl alcohol and the complementary production of chlorogenic acids in the growth culture of *Streptomyces albogriseolus* KF977548 isolated from decaying wood residues. Process Biochem., 52:22–29.

Bradbury JH, Egan SV, Lynch MJ. (1991). Analysis of cyanide in cassava using acid hydrolysis of cyanogenic glucosides. J Sci Food Agric., 55: 277-290.

Cereda MP. (2001). Caracterização dos subprodutos da industrialização da mandioca. *In*: Cereda, M.P. (ed.). *Manejo, uso e tratamento de subprodutos da industrialização da mandioca*. Fundação Cargill, São Paulo, p.13-37.

Cereda MP, Brasil. OG, Fioretto, AMC. (1981). Microrganismos com respiração resistente ao cianeto isolados de líquido residual de fecularia. *YTON*, 41:197-201.

Conn EE. (1969). Cyanogenic glycosides. J Agric Food Chem., 17:519-526.

Cooke RD, Blake GG, Battershill JM. (1978). Purification of cassava linamarase. Phytochem., 17:381-383.

Ernesto M, Cardoso AP, Cliff J, Bradbury JH. (2000). Cyanogens in cassava flour and roots and urinary thiocyanate concentration in Mozambique. J Food Composition Analys., 13:1–12.

Essers AJA, Bennink MHJ, Nout MJR. (1995). Mechanisms of increased linamarin degradation during solid-substrate fermentation of cassava. W J Microbiol Biotechnol., 11:266-270.

Fagbemi AO, Ijah UJJ. (2006). Microbial population and biochemical changes during production of protein-enriched fufu. W J Microbiol Biotechnol., 22:635-640

Formunyan RT, Adegbola AA, Oke OI. (1985). Technical note: the stability of cyanidrins. Food Chem., 17:221-225.

Jensen HL, Abdel-Ghafar AS. (1979). Cyanuric acid as nitrogen sources for microorganisms. Arch Microbiol., 67:1-5.

Jones DA. (1998). Why are so many plants cyanogenic? Phytochem., 47:155–162.

King NLR, Bradbury JHC. (1995). Bitterness of cassava: Identification of a new Apiosyl Glucoside and other compounds that affect its bitter taste. J Sci Food Agric., 68:223-230.

Knowles CJ, Bunch AW. (1986). Microbial cyanide metabolism. Advanc Microbial Physiol., 27:73-111.

Knowles CJ. (1976). Microorganisms and cyanide. Bacteriol Rev., 40:652-680.

Kobawila SC, Louembe D, Keleke S, Hounhouigan J, Gamba C. (2005).

Reduction of the cyanide content during fermentation of cassava roots and leaves to produce bikedi and ntoba mbodi, two food products from Congo. Afr J Biotechnol., 4 (7):689-696.

Kobawila SC, Louembe D, Keleke S, Traore AS. (2003). Aspects physico-chimiques et biochimiques de l fermentation des feuilles de manioc en ntoba mbodi. Procédés Biologique *et al*imentaire, 1:106- 119.

Legras JL, Jory M, Galzy P. (1990). Detoxification of cassava pulp using *Brevibacterium* sp. 312. Appl Microbiol Biotechnol., 33:529-533.

Louembe D, Kobawila SC.; Gisele Bounga Kalou, Keleke S. (2003). Etude microbiologique des feuilles fermentées de manioc: «ntoba mbodi». Tropiculturan, 21(3): 106-111.

Louembe D, Malonga M, Kobawila SC, Mavoungou O. (1997). Evolution de la teneur en composes cyanés des tubercules de manioc au cours du rouissage.- Activité linamarasique de bactéries lactiques. Microbiol Alim utr., 15:53-60

Malonga M, Mavoungou O, Keleke S, Kobawila SC, Louembe D. (1996). Aspects microbiologiques et biochimiques du rouissage du manioc. Microbiol Alim Nutr., 14: 73-81.

Malonga M, Mavoungou O, Kobawila SC, Louembe D, Brauman A. (1993). Les bactéries lactiques au cours du rouissage: caractérisation et évolution. Microbiol Alim Nutr., 11: 471-475.

Meyers PR, Gokoold P, Douglas DE, Woods DR. (1991). An efficient cyanide-degrading *Bacillus pumilus* strain. J Gen Microbiol., 137:1397-1400.

Murugan K, Yashotha, Sekar K, Al- Sohaibani1 S. (2012). Detoxification of cyanides in cassava flour by linamarase of *Bacillus subtilis* KM05 isolated from cassava peelfrican. J Biotechnol., 11(28):7232-7237.

Nartley F. (1968). Studies on cassava, Cyanogenesis: The biosynthesis of linamarin and lotaustralin in etiolated seedlings. Phytochem., 7:1307-1312.

Obayori OS, Ilori MO, Adebusoye SA, Oyetibo GO, Omotayo AE, Amund OO. **(**2009). Degradation of hydrocarbons and biosurfactant production by *Pseudomonas* sp. strain LP1. World J Microbiol Biotechnol., 25:1615–1623.

Okafor N, Ejiofor MAN. (1986). The microbial breakdown of linamarin in fermenting pulp of cassava (*Manihot esculenta* Crantz). Mircen J Appl Microbiol Biotechnol., 2: 327-338.

Palmisano MM, Nakamura LK, Duncan KE, Istock CA, Cohan FM. (2001). *Bacillus sonrensis* sp. nov., a close relative of *Bacillus licheniformis*, isolated from soil in the Sonoran Desert, Arizona. Int J Syst Evol Microbiol., 51:1671-1679.

Pitcher DG, Saunders NA, Owen RJ. (1989). Rapid extraction of bacterial genomic DNA with guanidine thiocyanide. Lett Appl Microbiol., 8:151-156.

Romero MC, Hammer E, Cazau MC, Arambarri AM. (2002). Isolation and characterization of biarylic structure–degrading yeasts: hydroxylation potential of dibenzofuran. Environ Poll., 118: 379-382.

Skowronski B, Strobel GA. (1969). Cyanide resistance and cyanide utilization by a strain of *Bacillus pumilus*. Can J Microbiol., 15:93–95.

Ugwuanyi JO, Harvey LM, McNeil B. (2007). Linamarase activities in *Bacillus* spp. Responsible for thermophilic aerobic digestion of agricultural wastes for animal nutrition. Waste Manag., 27:1501-1508.

Vasconcelos AT, Twiddy DR., Wesstby A, Reilly PJA. (1990). Detoxification of cassava during gari preparation. Int J Food Sci Technol., 25:198-203.

Vasconcellos SP, Cereda MP, Cagnon JR, Foglio MA, Rodrigues RA, Manfio GP, Oliveira VM. (2009). In vitro degradation of linamarin by microorganisms isolated from cassava wastewater treatment lagoons. Braz J Microbiol., 40(4):879–883.