Characterization of Cyanide Degrading Amylolytic Bacillus Species Isolated from Cassava Effluent

Fadahunsi, I.F*, Olalekan, A.A. & Ozabor, T.P.
[1]Applied Food Microbiology and Biotechnology Laboratory
Department of Microbiology
University of Ibadan
Ibadan, Nigeria.
***E-mail**: Sanmifadahunsi@yahoo.com

ABSTRACT

Cassava tubers are excellent source of carbohydrates which are cultivated extensively as a staple food in tropical regions. It is of high economic value and nutrient, however the presence of high cyanide content constitutes a major concern among cassava processors and consumers. Therefore, there is need to search for an efficient method to reduce cyanide content present to the recommended amount for human consumption. Thus, this research was designed to characterize bacteria isolated from cassava effluent with the ability to effectively hydrolyse starch and reduce cyanide present in cassava. Isolation and identification of cyanide degrading-amylolytic bacteria from cassava effluent was carried out using the culture dependent, phenotypic and molecular methods respectively. Growth in minimum medium, cyanide reduction efficiency, production of ammonia, linamarase and amylase activities, growth at different temperatures and pH were monitored spectrophotometrically. Eighteen Bacillus species were isolated, only four were non pathogenic and simultaneously utilized cyanide and hydrolyzed starch. The two isolates with the highest cyanide utilizing and starch hydrolyzing abilities were identified as Bacillus pumilus CN1 and Bacillus megaterium CN3. These Bacillus species grew well in minimum medium with an optimum growth of 0.676(optical density) and 1.200(optical density) at 72 hours respectively. They also exhibited cyanide removal efficiency of 98% and 99% respectively at 500ppm KCN at 96 hours of incubation. They produced considerable quantities of linamarase and amylase at optimum temperature of 370C and pH 8.0. In conclusion, the two isolates could be used as potential starter cultures in cassava fermentation for the production of cassava based foods.

Keywords: Cyanide, Linamarase, Amylase, Bacillus spp. Cassava, Effluent.

iSTEAMS Proceedings Reference Format
Fadahunsi, I.F*, Olalekan, A.A. & Praise, T.O. (2019): Characterization of Cyanide Degrading Amylolytic Bacillus Species Isolated from Cassava Effluent. Proceedings of the 18th iSTEAMS Multidisciplinary Cross-Border Conference, University of Ghana, Legon, Accra, Ghana. 28th – 30th July, 2019
Pp 203-218. www.isteams.net - DOI Affix - https://doi.org/ 10.22624/AIMS/iSTEAMS-2019/V18N1P24

1. INTRODUCTION

Cassava (Manihot esculenta Crantz) is a root crop consisting of starchy deposit which is a source of calories for more than 500 million people worldwide (FAO, 2000). It provides about 60% of the daily calorific requirements of Africa and Central America. It has been rated as the third most important food crop after rice and maize in the developing countries (Kobawila et al., 2005). Cassava is resistant to drought, pest, disease, and produces high yield under unfavourable environmental conditions (Edward et al., 2012). Nigeria is one of the leading producers of cassava in the world in excess of about 26 million tons (Onwuka and Ogbogu, 2007; Okpako et al., 2008) and over 95% of the harvested cassava is processed into foods for humans.

All morphological parts of the plant are valuable, the leaves are used for making soup and feed for livestock, while the stems are used for cultivation, mushroom production and cooking as firewood. In addition, the tuber is eaten fresh or processed into flour and can be used for the production of bio-fuel and industrial starch while the young leaves are consumed particularly in Africa because of its high protein content (McMahon et al., 1995; Achidi et al., 2005; Lebot, 2009) Nutritionally cassava tubers are made up 85-90% carbohydrate, 1.3% protein and high level of cyanide in the form of cyanogenic glucosides, linamarine and lotaustraline, which constitutes a major safety problem among cassava processors and consumers (Dunstan et al., 1996; Oyewole and Afolami, 2001; Nwabueze and Odunsi, 2007; Owuamanam et al.,2010). Long term consumption of substantial amount of cyanide has been implicated in the etiopathogenesis of serious health problems such as tropical ataxic neuropathy, Konzo- aspastic paraparesis of the leg, fibrocalculous pancreatic diabetes (FCPD), glucose intolerance coupled with iodine deficiency which leads to goitre and cretinism had been reported (Mathangi et al., 2000; Lambien et al., 2004; Kaewkannetra et al., 2009; Bandna, 2012). The toxicity of cyanide is achieved by attaching itself to cytochrome oxidase an important iron containing enzymes required for aerobic respiration in the living cells. The traditionally developed methods of processing cassava such as cooking, sun-drying, oven-drying and roasting have been reported to be inefficient in the removal of cyanogens present to safe level recommended for human consumption (Maopoog, 1989; Owuamanam et al., 2010; Murugan et al., 2012). In this study attempt was made to isolate bacteria from cassava effluent and characterize them for use as potential starter cultures in the fermentation/production of cassava based products.

2. MATERIALS AND METHODS

Collection of samples

Cassava effluent was collected from Orogun cassava processing plant located at Water corporation bus stop opposite University of Ibadan Nigeria in sterile container and transported to the Applied food microbiology laboratory University of Ibadan for subsequent analysis.

Isolation of bacteria from cassava effluent

The sample was serially diluted by transferring 10 ml into 90 ml of sterile distilled water and 0.1 ml of 10^{-4} dilution was inoculated into nutrient agar in sterile Petri dish using pour plate method of Harrigan and McCance (1966). Incubation was carried out at 37^0C for 24 hours. Representative colonies were selected and streaked to obtain pure isolates which were maintained on nutrient agar slants at -4^0C for further use.

Safety Evaluation

Gelatinase activity

This was carried out using the method described by Smith and Goodner (1959).

DNase

The DNAse agar (Oxoid, Wesel, Germany) was used to carry out this test. Spot inoculation of the test isolates was done and incubated at 37^0C for 4days. HCl (1M) was poured on the agar and a zone of clearance around the bacterial growth indicated a positive result (Deighton et al., 1988).

Heamolysis

A colony of each of the bacterial isolates was inoculated onto freshly prepared blood agar plates and incubated at 37^0C for 48 hours. Halos around the colonies indicated alpha-haemolysis or clear beta-heamolysis. The isolates that were not pathogenic or with safety properties were used for further study (Akinjogunla et al., 2014)

Isolation of cyanide-degrading bacteria.

The isolation procedure was carried out as described by Perumal et al. (2013). The two isolates with the highest cyanide degrading abilities were selected for further study.

Screening for Amylase producing bacteria
This was done according to the method described Thippeswamy et al. (2006). The two isolates with the highest cyanide degrading-amylolytic abilities were selected for further study.

Identification Procedure
The two isolates with the highest cyanide-degrading amylolytic activities were identified using phenotypic and molecular methods The Phenotypic methods considered the following parameters such as Gram staining, Spore stain, Indole test, Starch hydrolysis, Gelatin hydrolysis,Urease, Methyl red Motility, Catalase, Casein hydrolysis, Citrate, Oxidase and Sugar fermentation

Molecular identification
DNA extraction and PCR amplification of bacterial 16S rDNA were carried out using the methods described by Gomaa and Montaz (2006) while sequencing was done according to the method described by Hengstmann et al .(1999)

Phylogenetic analysis
Phylogenetic tree was generated by performing distance matrix analysis using the NT system. Database search and comparisons were done with the BLAST search using the National Center for Biotechnology Information (NCBI) database.

Growth of isolates in minimal media
This was done according to the method described Perumal et al. (2013). The pure bacterial isolates were grown in minimal medium (M9) which comprises of K_2HPO_4 (1.00g), KH_2PO_4 (3.40g), KCl (0.50g), $MgSO_4$ (0.50g), $FeSO_4$ (0.01g), Glucose (1%) and KCN in 1L of distilled water, pH adjusted to 7.0 and incubated in water bath set at 30^0C for 96hours. Samples were collected at 24 hours intervals and optical density values were read at 600 nm using UV spectrophotometer.

Cyanide degradation assay
Preparation of bacterial cell suspension
This was carried out according to the method of Atta et al.(2008)

Determination of cyanide concentration
The method Fisher and Brown (1952) was employed to determine cyanide concentration.

Determination of ammonia release
This was carried out according to the method described by Adjei and Ohta (1999).

Determination of linamarase activity
This was carried out according to the method described by Okafor and Ejiofor (1985)

Amylase enzyme extraction
The selected isolates were incubated at 37^o C for 24 hours in 50 ml of 8% (w/v) starch medium in 250 ml Erlenmeyer flasks, placed in a shaker incubator at 120 rpm. The extracellular enzyme solutions were obtained by centrifugation at 5000 rpm for 20 minutes using a high speed centrifuge. The supernatant obtained was collected and used for enzyme assay.

Amylase Assay
Amylase activity was carried out according to the method described by Bertrand et al. (2004).

GrowthatdifferentpH

The two organisms were grown in the previously described composed medium with pH adjusted to 4.0, 5.0, 6.0, 7.0, 8.0 and 9.0. Incubation was carried out at 30^0C for 48 hours and growth was determined using a spectrophotometer.

.

Growth at different Temperature

The two organisms were cultivated separately in the previously described composed medium and incubated at 25^0C, 30^0C, 35^0C and 37^0C for 48 hours and growth was determined using a spectrophotometer.

3. RESULTS

The result of the safety assessment of the bacterial isolates is shown in Table 1. All the eighteen bacterial isolates showed negative reactions to haemolysis, Dnase and gelatinase tests. The result of screening for cyanide degrading-amylolytic bacteria is shown in table 2. All the isolates utilised cyanide but only isolates CN1, CN3, CN4 and CN6 were potential amylase producers which is evident by exhibiting different zones of starch hydrolysis of 18, 15, 05 and 08mm respectively.

Table 1: Safety assessment of the bacterial isolates

Isolates code	Heamolysis test	DNase test	Gelatinase test
CN1	-	-	-
CN2	-	-	-
CN3	-	-	-
CN4	-	-	-
CN5	-	-	-
CN6	-	-	-
CN7	-	-	-
CN8	-	-	-
CN9	-	-	-
CN10	-	-	-
CN11	-	-	-
CN12	-	-	-
CN13	-	-	-
CN14	-	-	-
CN15	-	-	-
CN16	-	-	-
CN17	-	-	-
CN18	-	-	-

Table 2: Screening for cyanide degrading- amylolytic bacteria.

Isolate CN1 code	Cyanide utilization	Zone of hydrolysis(mm)
CN1	+	18
CN2	+	-
CN3	+	15
CN4	+	05
CN5	+	-
CN6	+	08
CN7	+	15
CN8	+	-
CN9	+	-
CN10	+	-
CN11	+	-
CN12	+	-
CN13	+	-
CN14	+	-
CN15	+	-
CN16	+	-
CN17	+	-
CN18	+	-

The results of phenotypic and 16S rRNA identification methods of CNI and CN2 are shown in tables 3a, 3b, figures 1a and 1b respectively. The phenotypic identification showed that the two isolates are B. pumilus and B.megaterium based on the biochemical characteristics and with reference to Bergey's manual of systematic bacteriology while16S rRNA confirmed their Identities and phylogeny of the isolates CN1 and CN3 as Bacillus pumilus SAFR-032 and Bacillus megaterium QM B1551 respectively.

The strains showed maximum similarity ratio towards Bacillus pumilus SAFR-032 (99%) accession number NC_074977.1 and a similarity of 99% identity with the sequence of 16S rRNA of Bacillus megaterium QM B1551 accession number NC_074290.1 respectively using NCBI data base and hence the isolates were named as B. pumilus CN1 and B. megaterium CN3. The phylogenetic tree was calculated using the neighbour-joining algorithm.

Table 3a. Morphological, biochemical and physiological properties of cyanide-degrading bacteria isolated from cassava effluent

Isolate codes	Colony morphology	Cell morphology	Catalase test	Gram stain	Spore stain	Vp	Oxidase	Growth at 45^{0}C	Methyl red	6.5 NaCl	Motility	Indole	Argine hydrolysis	Casein hydrolysis	Starch hydrolysis	Gelatin	Urease	Propable Identity
CN 1	Small, white	Rod	+	+	+	-	-	+	+	+	+	-	-	+	+	-	-	Bacillus pumilus
CN 3	Large, white	Rod	+	+	+	-	-	+	+	+	+	-	-	+	+	-	+	Bacillus megateriu m

Table 3b. Sugar fermentation pattern of CN1 and CN3

Isolate codes	Glucose	Fructose	Sucrose	Maltose	Mannose	Galactose	Xylose	Arabinose	Inositol	Inulin	Sorbitol	Lactose	Raffinose	Mannitol	Propable Identity
CN1	+	+	+	+	+	-	+	+	-	-		-	+	+	Bacillus pumilus
CN3	+	+	+	+	+	-	+	+	-	-		-	+	+	Bacillus megaterium

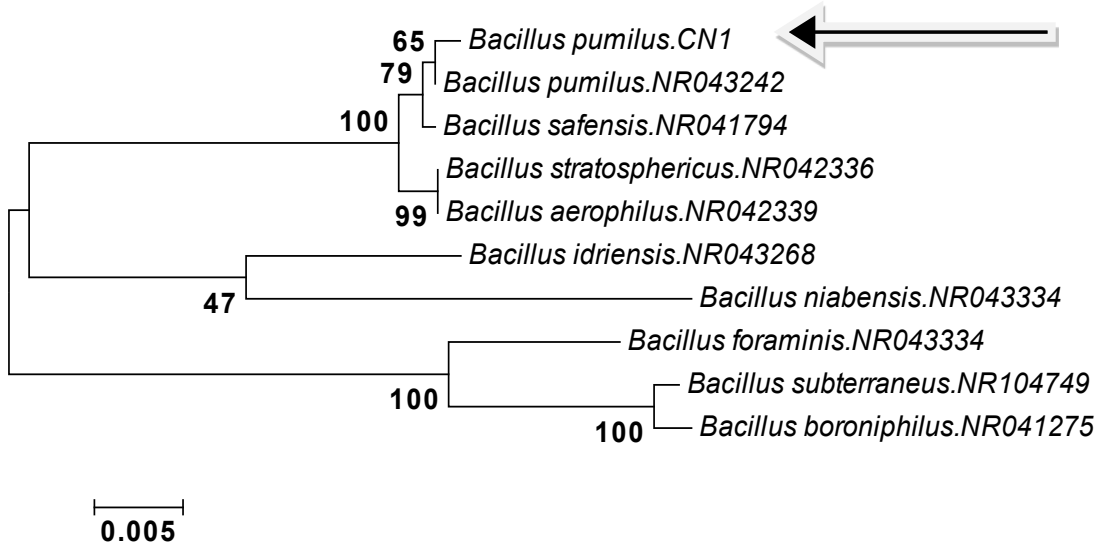

Figure 1a. Phylogenetic showing the evolutionary relationship of B. pumilus CN1 to closely related sequences obtained from the GenBank using MEGA 5.

❖ Multiple alignments of the sequences corresponding to the 16S rRNA of the studied isolate were carried out followed by neighbour joining clustering. Bootstrap values expressed as percentages of 1000 replications.

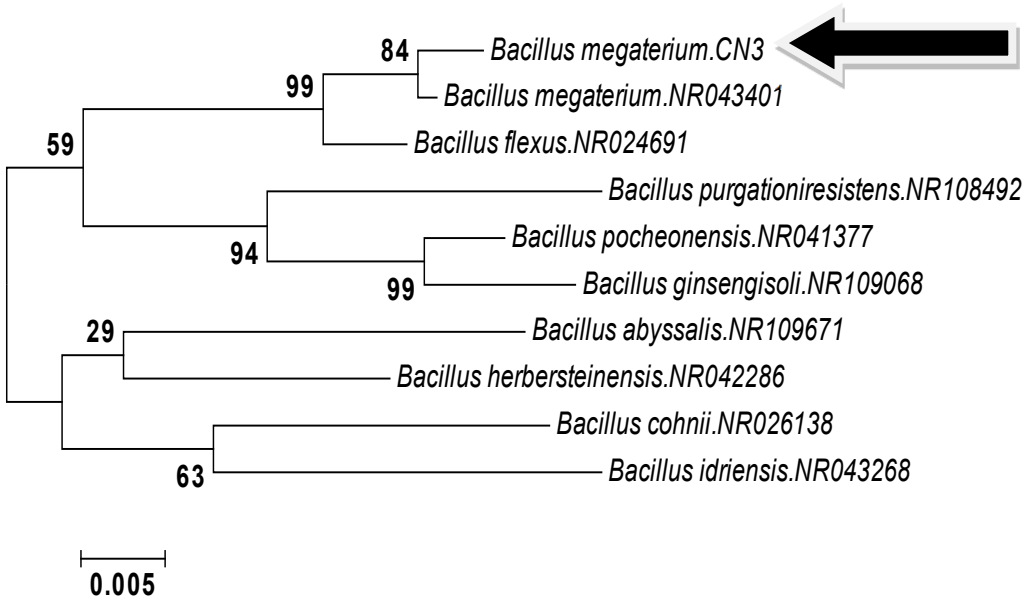

Figure 1b: Phylogenetic showing the evolutionary relationship of B. megaterium CN3 to closely related sequences obtained from the GenBank using MEGA 5.

❖ Multiple alignments of the sequences corresponding to the 16S rRNA of the studied isolate were carried out followed by neighbour joining clustering. Bootstrap values expressed as percentages of 1000 replication

The growth pattern of B. pumilus CN1 and B. megaterium CN3 in minimal medium is shown in Figure 2. The two microorganisms grew well in minimum medium with B. pumilus CN1 showing an optimum growth of 0.676 (OD) at 72 hours which decreased to 0.532(OD) at 96 hours while B. megaterium CN3 had a maximum growth of 1.200 (OD) at 72 hours which decreased to 0.960 (OD) at 96 hours.

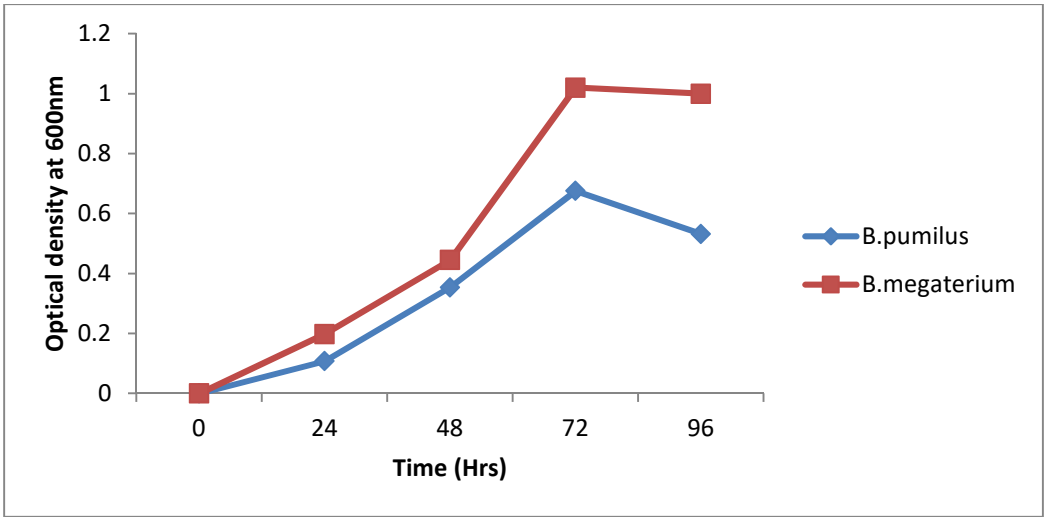

Figure 2: Growth pattern of B. pumilus CN1 and B. megaterium CN3 in minimal medium

The results of Cyanide removal efficiency (Cyanide tolerance) for B. pumilus CN1 and B. megaterium CN3 at 96 hours of incubation in minimal medium with KCN (25, 50, 150 and 500ppm) as the sole nitrogen source are shown in Figures 3a, 3b, 3c, 3d. It was observed that at 24 hours period of incubation B. pumilus CN1 recorded 70% removal while B. megaterium showed 77% removal efficiency. However, at 96 hours of incubation B. Pumilus and B. megaterium recorded 96.8% and 98% removal efficiencies respectively (Fig 3a). B. pumilus showed removal efficiency of 71% after 24 hours which increased to 97% at 96 hours of incubation while B. megaterium recorded 79% and 98% removal efficiencies respectively at 24 and 96 hours of incubation respectively (Fig 3b). Figure 3c shows the removal efficiency at 150 ppm. It was also noted that B. pumilus exhibited 87% removal efficiency at 24hr which increased to 97% removal efficiency at 96 hours while 89% and 99% were recorded for B. megaterium at 24 and 96 hours of incubation periods respectively. The result for removal efficiency of B. pumilus CN1 and B. megateriumCN3 at 500ppm is shown in Figure 3d. B. pumilus CN1 was able to remove 86% of KCN present while B. megaterium CN3 was able to remove 88% at 24 hours of incubation. 98% and 99% removal efficiency was recorded for B. pumilus CN1 and B. megateriumCN3 respectively at 96 hours of incubation

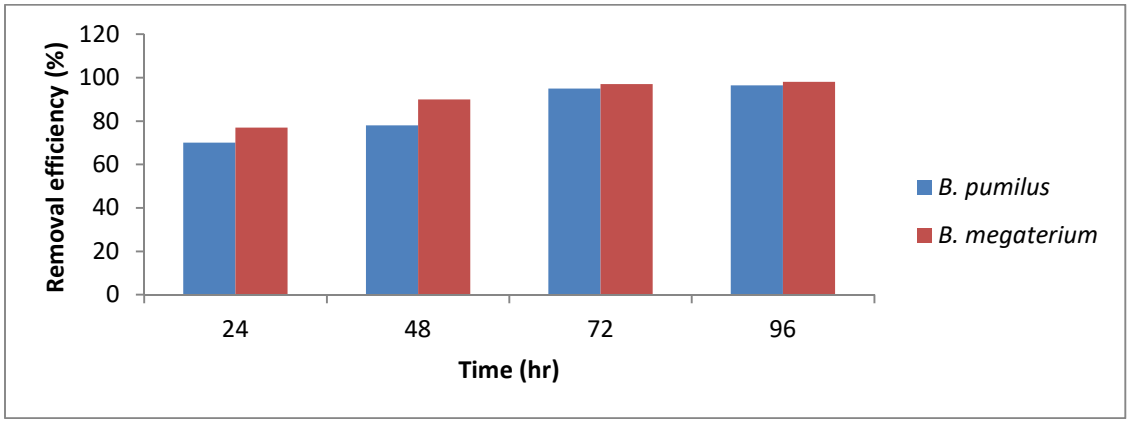

Figure 3a: Cyanide removal efficiency for B. pumilus CN1 and B. megaterium CN3 at 25ppm

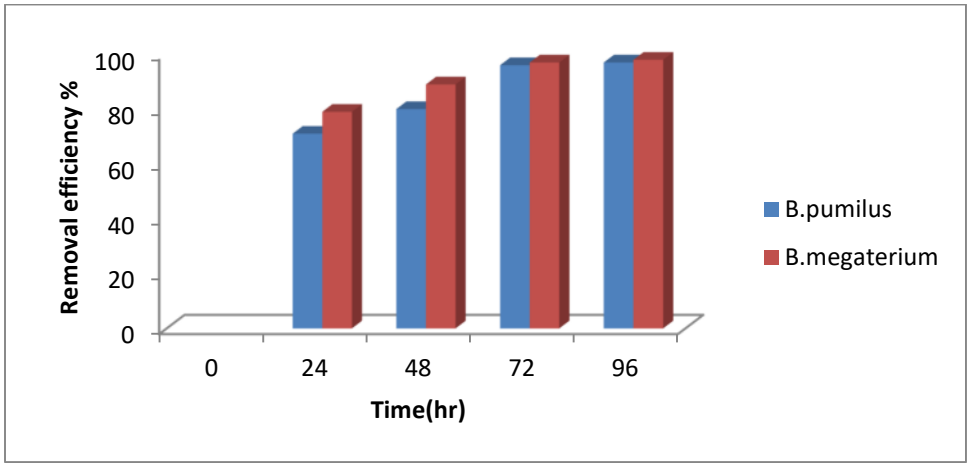

Figure 3b: Cyanide removal efficiency of B. pumilus CN1 and B. megaterium CN3 at 50ppm

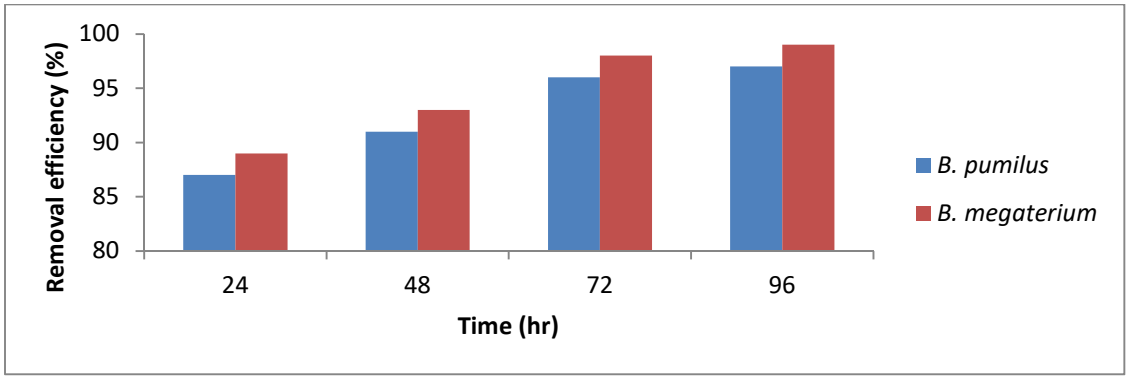

Figure 3c: Cyanide removal efficiency of B. pumilus CN1 and B. megaterium CN3 at 150ppm

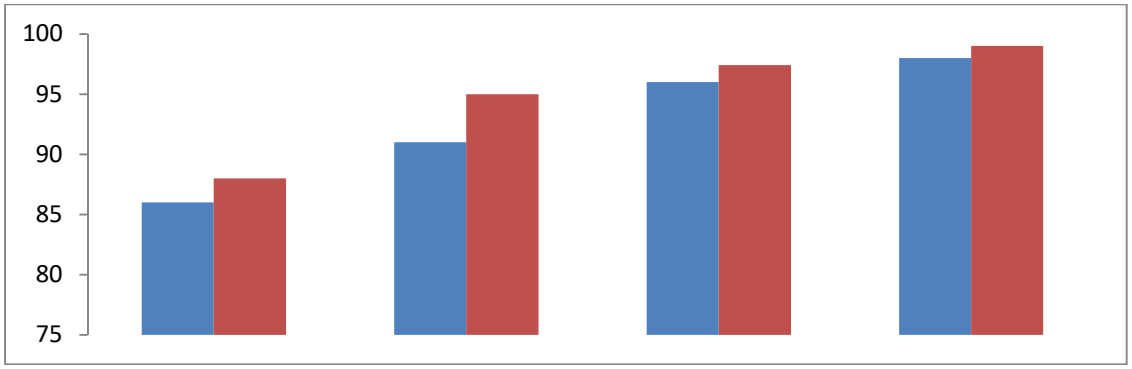

Figure 3d: Cyanide removal efficiency of B. pumilus CN1 and B. Megaterium CN3 at 500ppm

The quantities of ammonia released during cyanide degradation by B. pumilus CN1 and B. megaterium CN3 is depicted in figure 4. The quantity of ammonium produced by B.megaterium at 24 and 96 hours was 0.134mg/ml and 1.298mg/ml respectively while B. pumilus produced 0.089mg/ml and 1.012mg/ml at 24 and 96 hours respectively. Figure 5 shows the linamarase activities of B. pumilus CN1 and B. megaterium. It was observed that the two species of Bacillus had linamarase activities with B. megaterium CN3 producing the higher activity of 7.2 µmol/ml at 72 hours which decreased to 6.3 µmol/ml at 96 hours while B.pumilus produced an activity of 6.4 µmol/ml at 72 hours which decreased to 5.2 at 96h µmol/ml.

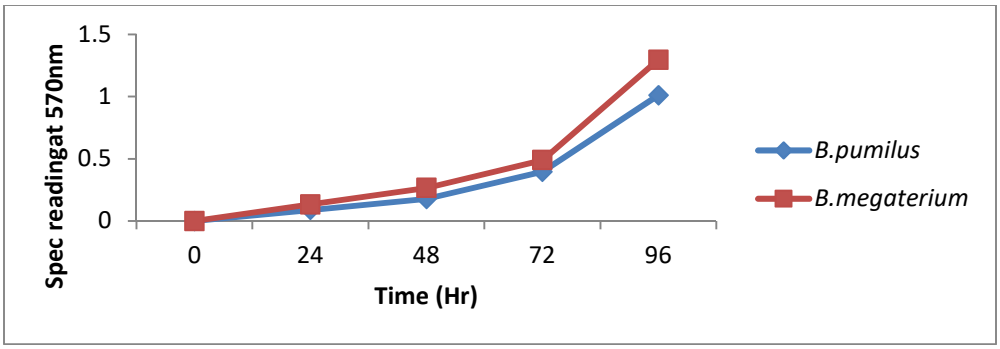

Figure 4. Ammonia release during cyanide degradation by B. pumilus CN1 and B. megaterium CN3.

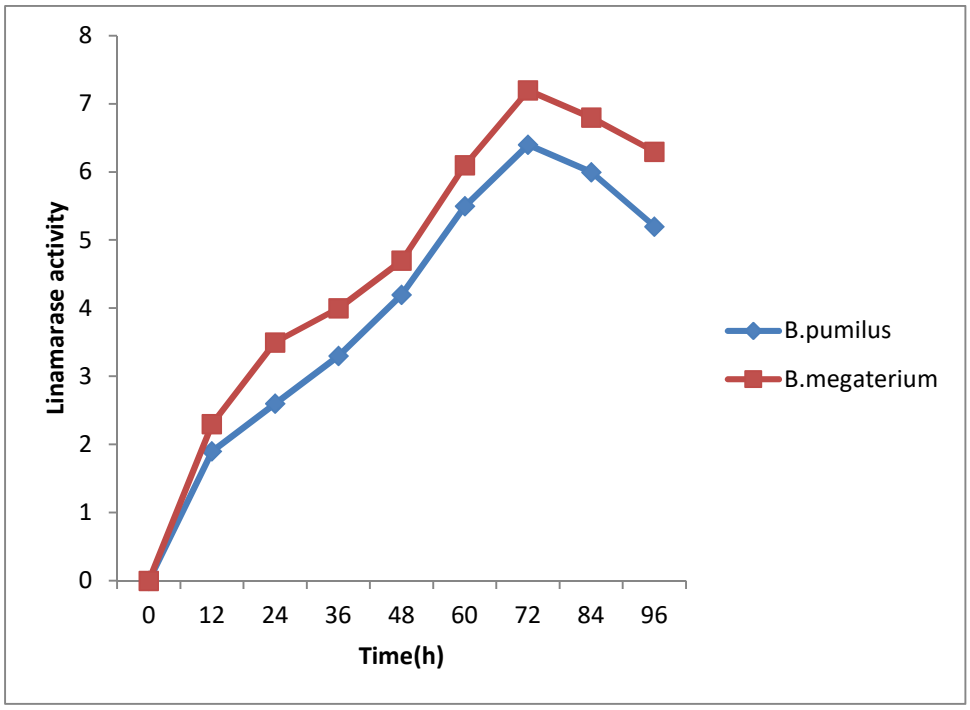

Figure 5: Linamarase activities of B. pumilus CN1 and B. megaterium

The amylase activities of two Bacillus species are presented in Figure 6. It was revealed that two Bacillus species had amylase activities with B. megaterium producing its optimum activity of 6.2µmol/ml at 72 hours which decreased ton4.7 µmol/ml at 96 hours while B.pumilus produced its optimum of 5.8 µmol/ml at 72 hours which decreased to 4.2 µmol/ml at 96 hours.c

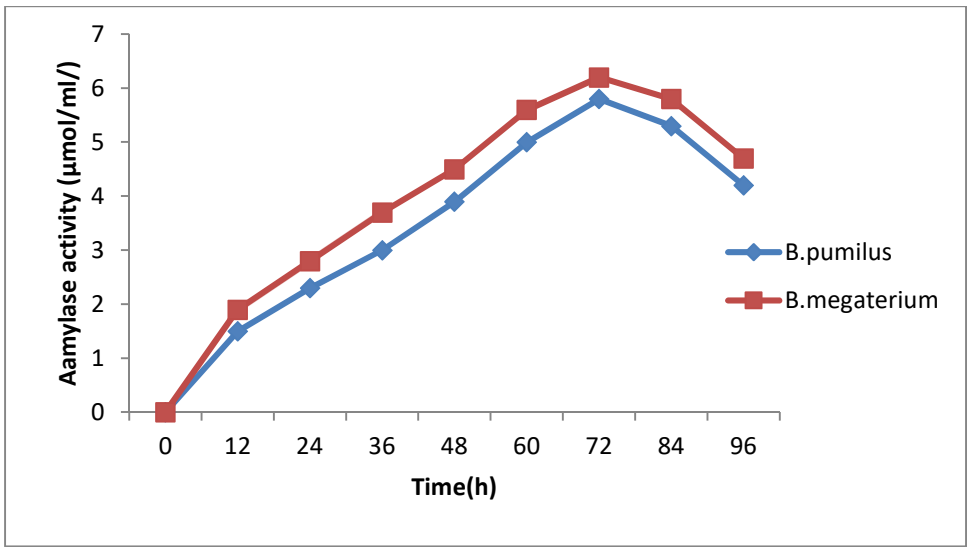

Figure 6: Amylase activities of B. pumilus CN1 and B. megaterium CN3

The result of growth pattern of the two Bacillus species at different temperatures is shown in figure 7. B. megaterium and B. pumilus produced their optimum growth of 0.39 and 0.35 respectively at 37 ^{0}C

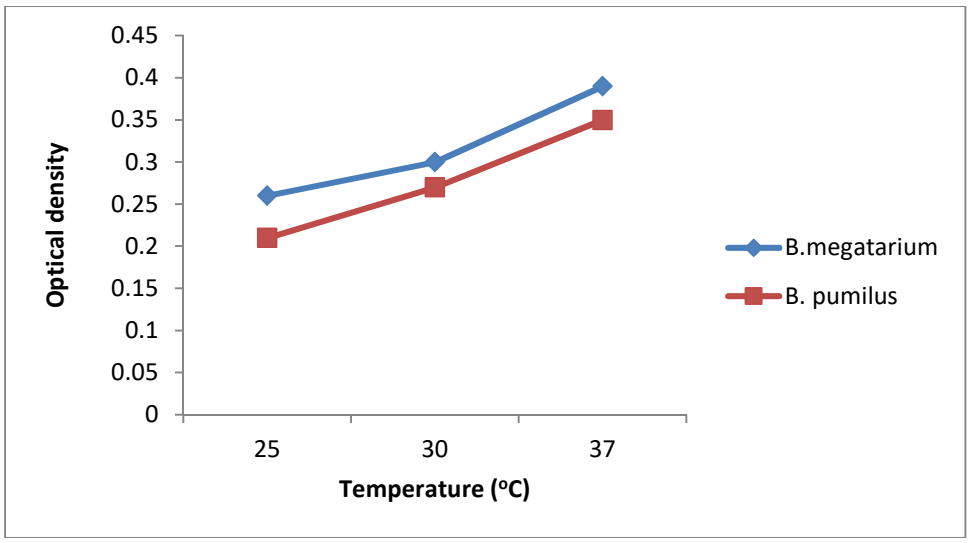

Figure 7: Growth Pattern of B. pumilus CN1 and B. megaterium at different

Temperatures

Figure 8 shows the growth pattern of the two Bacillus species at different pH. B. pumilus and B. megaterium had their maximum growth of 0.58 and 0.51 respectively at pH 8.0.

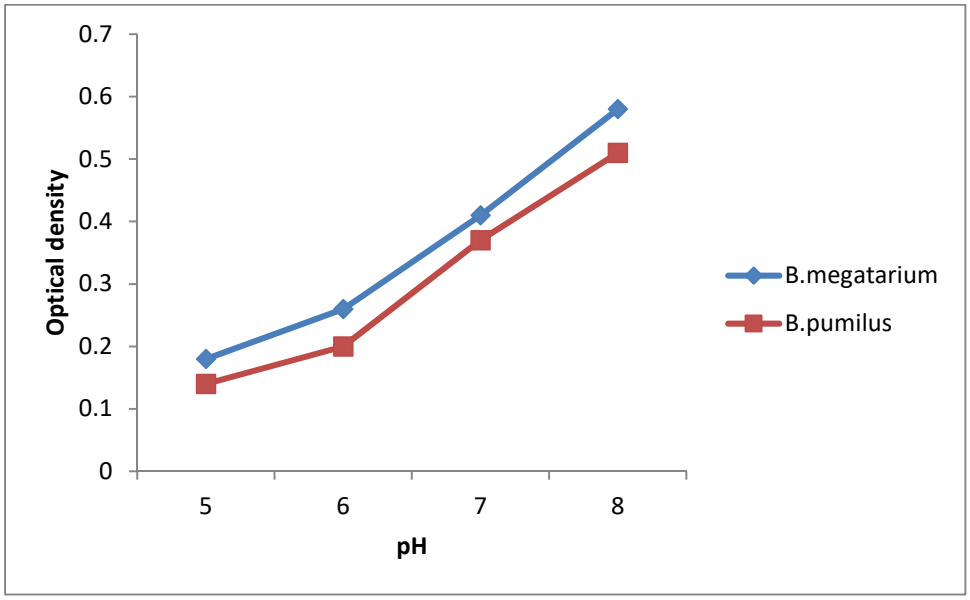

Figure 8: Growth Pattern of B. pumilus CN1 and B. megaterium at different pH

4. DISCUSSION

The isolated bacteria from cassava effluent were identified using phenotypic and molecular methods as Bacillus pumilus and Bacillus megaterium. Full and partial 16S rRNA gene sequencing methods has been reported as a more objective, accurate, and reliable method for bacterial identification. In addition it possesses the capability of confirming taxonomic relationships among bacteria (Pretti et al., 2005) and identifying phenotypically aberrant microorganisms. The isolation of Bacillus species from cassava effluent had earlier been reported by Dash et al.(2009) ; Dursun et al.(2009) ; Kumar et al.(2013); Wu et al.(2013). The survival of Bacillus species in cyanide contaminated environment is due to their ability to grow and tolerate cyanide toxicity. There is the possibility of these microorganisms making use of the cyanide in this environment as a source of nitrogen for growth and metabolism. Perumal et al. (2013) had earlier reported the isolation of two Bacillus species in a minimal medium containing cyanide as the only nitrogen source which survived higher concentration of cyanide and grew in 6000mg/L and 7000mg/L of KCN, while Skowronski & Strobel (1969) isolated a strain of Bacillus pumilus which could survive in solutions containing 2.5 M KCN and grew in media containing KCN (2600mg CN) In addition, Sartry (1986) reported the isolation of some cyanide-metabolizing bacteria such as Bacillus megaterium, B. pumilus, B. cereus, and B. Stearothermophilus from cassava effluent.

The ability of an organism to hydrolyse starch is an indication of a potential amylase producer. The two Bacillus species selected in this study were highly amylolytic which is evident from the wide zone of clearance they produced. This observation was similar to the earlier report of Anto et al. (2006). The tolerance and degradative ability of the organisms were monitored by quantifying growth, cyanide degradation and ammonia released. The production of large quantity of ammonia coupled with the growth of the organisms showed that the organisms can tolerate high level of cyanide. Fisher et al. (2000) had earlier reported similar finding. In this study it was observed that the quantity of ammonia released increased as the time of degradation increased or removal efficiency increased. This occurrence is in agreement with previous submissions which reported that free cyanide disappearance was accompanied by a concomitant increase in ammonia as one of the end-products of aerobic and anaerobic degradation of cyanide. The ability of an organism to metabolize cyanide is dependent on factors such as possession of a biodegradable pathway to convert cyanide into an assimilative product ($NH4^{+}$), cyanide resistance mechanism and a system for taking up Fe^{3+} from the medium.

The presence of these pathways in organisms confers on them the ability to be used in the development of biotechnologies to degrade cyanide compounds or convert cyanide into ammonium (Ebbs, 2004; Luque-Almagro et al., 2005). In addition the degradation of cyanogenic glycosides contains a series of steps, such as the splitting of the sugar moieties from the aglycone/cyanohydrin by β-glycosidases, followed by the degradation of the cyanohydrin into a carbonyl compound and hydrogen cyanide (Vetter, 2000). It is evident from this study that both B. pumilus CN1 and B. megaterium CN3 were able to degrade cyanide and produce ammonia and this is in tandem with the earlier report of Peruma et al. (2013).

The safety assessment revealed the suitability of the bacteria as starter culture for food fermentation. The use of pathogenic organisms as starter cultures in food fermentation can lead to public health challenge for consumers. Linamarase and amylase activity are important factors in the selection of organism for cyanide degradation. The bacterial isolates had high amylase activity which is responsible for their starch hydrolysing activity. This observation is in conformity with earlier reports of Oyewole and Odunfa, (1990) ; Kimaryo et al. (2000); Anto et al. (2006) which confirmed the presence amylases in microorganisms. Pandey et al. (2000) described amylases as extracellular enzymes that randomly cleave the 1, 4-a-D-glucosidic linkages between adjacent glucose units in the linear amylose chain. Bacillus species had been reported as the most important sources of amylase and have been employed for amylase (enzyme) production in laboratory study and industrially (Anto et al., 2006). The result of amylase activity reported in this work is in conformity with the previous findings of Thippeswamy, et al. (2006); Rasooli et al. (2008); Oyeleke et al.(2010); Sethi et al (2013).

In this study, it is evident that there was considerable linamarase production. The two Bacillus spp. produced linamarase which is an indication of their ability to degrade cyanide. This observation corroborates the earlier submissions of Oyewole and Odunfa, (1990), Kimaryo et al. (2000), Cumbara et al. (2007) which emphasised the significance of linamarase enzyme in cyanide degradation. In addition, Okafor and Ejiofor (1985); Oyewole and Odunfa (1990); Kimaryo et al. (2000); Ahaotu et al. (2013) reported that linamarase producing microorganisms are useful in the liberation of cyanide from grated cassava during gari production. The optimum growth of the two organisms was seen at high pH which is an indication that they were alkaline in nature and could be suitable for alkaline fermentation. This observation is in conformity with the report of Maegala et al. (2011).

Temperature is an essential factor for growth and metabolism of microorganisms (Maegala et al., 2012). The temperature of natural fermentation of cassava falls within the growth temperature range of the Bacillus spp. therefore, it can be inferred that they could be employed for the fermentation of cassava for the production of cassava based food products.

5. CONCLUSIONS

From the empirical results obtained from this study it could be concluded that the two Baciilus spp. demonstrated high cyanide degrading and starch hydrolysing abilities. Hence, they could be used as potential starter cultures in the fermentation of cassava and production of cassava based products with reduced cyanide and starch contents.

REFERENCES

1. Achidi A.U., Ajayi, O. A., Bokanga M., Maziya-Dixon B. (2005). The use of cassava leaves as food in Africa. Ecology of Food and Nutrition. **44**: 423–435.
2. Adjei M., Ohta M.Y. (1999). Isolation and characterization of a cyanide-utilizing Burkholderia cepacia strain. World Journal of Microbiology and Biotechnology. **15:** 699 – 704.
3. Ahaotu I., Ogueke, C.C., Owuamanam C.I., Ahaotu N.N., Nwosu, J.N. (2013). Fermentation of undewatered cassava pulp by linamarase producing microorganisms:effect on nutritional composition and residual cyanide. American Journal of Food Nutrition **31:** 1-8.
4. Akinojgunla, O.J., Ajayi A.O., Eket, N.O. (2014). Virulence factors and antibiotic resistant species from the anterior nares of apparently healthy undergraduate in Uyo.American journal of research communication. 2(11) 158-180.
5. Anto H., Trivedi U., Patel K . (2006). Alpha amylase production by Bacillus cereus MTCC 1305 using solid state fermentation. Food Technol Biotech .44 241-245
6. Atta M.B., Sabet K.K., Mostafa H.E., Rizk A.M. (2008). Characterization of Cyanide-degrading bacteria isolated from industrial wastewater. Journal of food science and Technology. Special volume conference: 91-97
7. Bandna C. (2009). Effect of processing on the cyanide content of cassava products in Fiji. Journal of Microbiology, Biotechnology and Food Sciences. **2.3:** 947-958
8. Bertrand T.F., Frederic, T. Robert, N. (2004). Production and partial characterization of a Thermostable Amylase from Ascomycetes yeast strain isolated from starchy sail. McGraw Hill Inc., New York, USA, pp: 53 – 55.
9. Dash R.R., Gaur A., Balomajumder C. (2009). Cyanide in industrial waste waters and its removal: a review on bio-treatment. Journal of Hazard Materials.**163**: 1– 11
10. Cumbara A., Mirione E., Cliff J., Bradbury J.H. (2007).Reduction of cyanide content of cassava flour in Mozambique by the washing method . Food Chem. 101(3):894-897
11. Deighton M.A., Flanklin J.C., Spoker W.J., Walker, B. (1988). Species identification, antibiotic sensitivity and slime production of coagilase negative Staphylococcus isolated from clinical sample. Epidemiology infection 101:99-113
12. Dunstan W.R., Henry T. A., Auld S.J.(1996). Cyanogenesis in plant. The occurrence of phaseolunatin in cassava Manihot aipi and Manihot utilissima. Procedure of Royal. Document London. **78:** 152-158.
13. Dursun A.Y, Calik, A., Aksu, Z. (2009). Degradation of ferrous (II) cyanidecomplex ion by Pseudomonasflourescens. Process Biochemistry. **34**: 901-908.
14. Ebbs S. (2004). Biological degradation of cyanide compounds. Current Opinion in Biotechnology. **15:** 231-236
15. Edward V.A., Egounlety M., Huch M., Petrus, J., Singh S., Naledzani D. Vetja M. Charles M.A. (2012). Isolation and screening of microorganisms from a gari fermentation process for starter culture development. African Journal of Biotechnology. **11.65:** 12865-12877
16. FAO (2000). Food and Agriculture Organization of the United Nations. Championing the cause of cassava. File /A./Championing cause of cassava.
17. Fisher F B., Brown J.S**. (**1952). Colorimetric determination of cyanide in stack gas and waste water. Analytical Chemistry**. 24:** 1440- 1444.
18. Gomaa O., Momtaz O. (2007). 16S rRNA characterization of a Bacillus isolate and its tolerance profile after subsequent subculturing. Arab Journal of Biotechnology. **101:**107-116.
19. Harrigan W.F., McCance M.E. (1966). Laboratory Methods in Microbiology. Academic Press, London, UK.
20. Hengstmann U., Chin K., Janssen P.H. Liesack, W. (1999). Comparative phylogenetic assignment of environmental sequences of genes encoding 16S rRNA and numerically abundant culturable bacteria from anoxic rice paddy soil. Applied Environmental Microbiology **5(11):** 5050-5058.

21. Kaewkannetr P., Imai T., Garcia-Garcia F.J., Chiu, T.Y. (2009). Cyanide removal from cassava mill wastewater using Azotobactor vinelandii TISTR 1094 with mixed microorganisms in activated sludge treatment system. Journal of Hazardous Materials **172(1):** 224-228.
22. Kimaryo V.M., Massawe G.A., Olasupo N.A., Holzaphel W.H. (2000). The use of a starter culture in the fermentation of cassava for the production of Kivunde, a traditional tamanian food product. International Journal of Food Microbiology. **56:** 179-190.
23. Kobawila S.C., Louembe D., Keleke M.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 African Journal of Biotechnology. **47:** 689-696
24. Kumar, V. and Bhalla, T. C. 2013. In vitro cyanide degradation by Serratia marcescens RL2b. International Journal of Environmental Science.**3**: 1985-1995
25. Lambien F., Defoort B., Kuo Y. (2004). Konso or Neurolathyrism: Is there a difference? Cassava Cyanide Disease Network News. **3:** 2-3.
26. Lebot V. (2009). Tropical root and tuber crops: cassava, sweet potato, yams and aroids. Centre of Agriculture and Bioscience International. **5**: 234-345
27. Luque-Almagro V.M., Blasco R., Martinez-Luque M., Moreno-Vivian, C., Castillo, F., Roldan, M. D. (2005). Bacterial cyanide degradation is under review: Pseudomonas psedoalcaligenes CECT5344, a case of an alkaliphilic cyanotroph. Biochem. Soc. Trans. **391:** 269-74.
28. Maegala N.M., Fridelina, S. Abdullatif, I. (2011). Biodegradation of cyanide by Rhodococcus strains isolated in Malaysia. International Conference for Food Engineering and Biotechnolog. **9**: 21–25.
29. Maopoog O., Chism G., Sayre R. (1989). Isolation of cassava linamarase: evidence that endogenous levels are insufficient for effective hydrolysis of linamarin. Report on the founding workshop for the Founding for the Cassava Biotechnology Research Network , CIAT document No 52, p,25
30. Mathangi D. C., Deepa R., Mohan, V. Govindarajan, M., Namasivayam, A. (2000). Long term ingestion of cassava tapioca does not produce diabetes or pancreatitis in rat model. International Journal of Pancreatology. **27:** 203-208.
31. McMahon J.M., White W.L., Sayre R.T. (1995). Cyanogenesis in cassava Manihot esculanta Crantz. Journal of Experimental Botany. **46:** 731-741
32. Mkpong O.E., Hua-Yan E., Chism G.,Sayre, R.T.(1990). Purification, characterization and localization of linamarase in cassava. Plant Physiol. **93:** 176 - 181.
33. Murugan K., Yashotha K., Saleh Al- Sohaibani. (2012). Detoxification of cyanides in cassava flour by linamarase of Bacillus subtilis KM05 isolated from cassava peel. African Journal of Biotechnology. **11(28):** 7232-7237
34. Nwabueze TU., Odunsi, F.O (2007). Optimization of process conditions from cassava Manihot esculenta Lafun production. African Journal Biotechology. **65:** 603-611.
35. Okafor N., Ejiofor, M.A. (1985). The linamarase of Leuconostoc mesenteroides, production, isolation and some properties. Journal Science of Food Agriculture. **36:** 667-678.
36. Okpako, C.E., Ntui, V.O., Osuagwu, A.N., Obasi FI. (2008). Proximate composition and cyanide content of cassava peels fermented with Aspergillus niger and Lactobacillus rhamnosus. Journal of Food Agriculture. **62:** 251 – 255.
37. Onwuka G.I., Ogbogu N.J. (2007). Effects of fermentation on the quality and physicochemical properties of Cassava based Fufu products made from two Cassava varieties NR8212 . Journal of Food Technology. **5 (3):**26 – 264
38. Oyeleke SB., Auta SH., Egwin E.C (2010). Production and characterization of amylase produced by Bacillus megaterium isolated from a local yam peel dumpsite in Minna Niger state. African journal of biotech. 2(7) :88-89
39. Oyewole, O. B. and Odunfa, S. A. (1990). Characterization and distribution of lactic acid bacteria in cassava fermentation during fufu production. Journal of Applied Bacteriology. **68:** 145-152.

40. Oyewole O.B., Afolami O.A. (2001).Quality and Preference of different cassava varieties for Lafun production. Journal of Food Technology in Africa **6:** 27-29.
41. Owuamanam J.O., Iwouno N., Ihediohanma C. Barber L. (2010). Cyanide Reduction, Functional and Sensory Quality of Gari as Affected by pH, Temperature and Fermentation Time. Pakistan Journal of Nutrition. 9(10): 980-986.
42. Pandey A., Nigam P, Soccol C.R., Soccol, V. T., Singh D., Mohan, R. (2000). Advances in microbial amylases. Biotechnology and Applied Biochemistry. **31:** 135–152.
43. Perumal M., Prabakaran, J. Kamaraj M. (2013). Isolation and Characterization of Potential Cyanide Degrading Bacillus nealsonii from Different Industrial Effluents. International Journal of Chem Tech Research. **55:**2357-2364.
44. Petti C.A., Polage C.R., Schreckenberger P. (2005). The Role of 16S rRNA Gene Sequencing in Identification of Microorganisms Misidentified by Conventional Methods. Journal of Clinical Microbiology. **43(12):** 6123-6125.
45. Rasooli I., Astaneh S.D., Bomett , K.A. (2009). A Thermostable Alpha amylase producing natural variant of Bacillus spp .isolated from soil in Iran. Am. J. Agric Biology Sci. 3(3):591-596
46. Sastry, C.A(1986). Industrial waste biodegradation. Encology.7:19-24. 35.
47. Sethi S Nanda R Chakraborty T (2013). Clinical application of volatile organic compound analysis for detecting infectious diseases Clin Microbiol Rev. 26: 462-75
48. Skowronshi B., Strobel G.A. (1969). Cyanide resistance and cyanide utilization by a strain of B.pumilus .Canadian Journal of Microbiology. 15:93-8
49. Smith J.L., Goodner K. (1959). Detection of bacterial gelatinase by gelatine- agar plate method. Journal of Bacteriology. 76(6):662-5
50. Thippeswamy S., Lokesh V., Ravishankar R. (2006). Relationship between essential oil treatment on seed mycoflora and seedling quality of some oil seed crop species. Asian. Journal of Microbial biotechnology and Environmental. **4**: 439-443
51. Vetter J. (2000). Plant cyanogenic glycosides. Toxicon **38:**11–36.
52. Wu C.F., Feng A.J., Xu X.M., Huang S. H., Deng M.C. (2013). Construction of recombinant Pichia strain GS115-Ch-Glu expressing ß-glucosidase and cyanide hydratase for cyanogenic glycosides detoxification. African Journal Biotechnology. **11**: 4424-4423.