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Molecular Characterization and Potential of Bacterial Species Associated with Cassava Waste

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ABSTRACT

Knowledge of the true microbial diversity in cassava waste (CW) is fundamental to effective utilization of this waste. This paper reports, on the identification of bacteria species associated with CW, using molecular tools. The 16S rRNA gene of total bacteria community and bacterial isolates were amplified by Polymerase Chain Reaction (PCR) using 16S rRNA primers. Total microbial community DNA amplicons were spliced into the PCR-TRAP Cloning Vector, used to transform competent cells of *Escherichia coli* and sequenced. Sequences were identified by aligning with sequences in the GenBank. Twenty four bacterial species were detected in cassava peel (CP) belonging to *Bacillus-Bacillales* (7 species), *Bacillus-Lactobacillales* (12 species), Gamma-proteobacteria (3 species), *Acinetobacteria-Actinomycetales* (1 species) and uncultured bacteria (2 species). *Bacillus licheniformis* (11.3%) and *B. substilis* (11.3%) were the dominant species. *Azotobacter vinelandii*, an uncultured bacterium clone ncd1462c07c1 and an uncultured compost bacteria clone PS2630 identified in this study, probably has not been reported in cassava fermentation. In cassava wastewater (CWW), 26 bacterial species were detected including *Bacillus-Bacillales* (5 species), *Bacillus-Lactobacillales* (18 species), Gamma-proteobacteria (2 species), *Acinetobacteria-Actinomycetales* (1 species), Beta-proteobacteria (1 species) and uncultured bacteria (1 species). *Lactobacillus fermentum* (11.1%) was the dominant species closely, followed by *L. plantarum* (10.7%). The potential of some of the species are highlighted. This study has shown that CW is an important microbial resource, considering its rich bacterial diversity.

Keywords: Cassava waste, molecular characterization, community DNA, 16S rRNA, cloning.

Introduction

Cassava process wastes, including peels, fibrous core and the carbohydrate rich pressing slurry, account for over 50% of the tuber on a wet weight basis (Adeneye and Sunmonu, 1994). Consequently, a large amount of cassava waste (CW) is generated annually. Hou *et al*. (2007) estimated that about 0.3 – 0.4 tonne of cassava peel (CP) is generated when 1 tonne of starch is produced. It seems likely that even more wastewater is generated during processing of cassava. These wastes generated pose serious environmental challenges at present and would even create more problems in the future, especially with ongoing effort by the Nigerian government to stimulate cassava production and utilization, which has led to increased industrial production of cassava products such as cassava flour, industrial starch and dried cassava "fufu" among others. Although domestic animals such as pigs, ruminants and poultry may feed on the peels, its use for this purpose is often limited, ultimately, by the high level of toxic cyanogenic glycosides which may constitute a health hazard to the animals.

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Considerable research has been conducted and is currently being intensified to maximize the use of CW for value added products. The production of animal feed from CP has been well studied (Iyayi and Lossel, 2000; Tweyongyere and Katongole, 2002; Akinfala and Tewe, 2004). A major improvement in this regard, is the nutrient enrichment of CP by fermentation using microorganisms such as yeast and lactic acid bacteria (Oboh, 2006), *Aspergillus* and *Trichoderma* species (Obadina *et al.*, 2006), for enhanced animal nutrition. CP has also been used for the production of functional food (Raupp *et al.*, 2004), ethanol (Adesanya *et al*., 2008), biofertilizer (Ogbo, 2010), and as substrate for mushroom cultivation (Beux *et al*., 1995). Similarly, cassava wastewater (CWW) has been used for the production of butanol (Wang *et al*., 2012), organic acids (Pandey *et al*., 2007), biosurfactant (Nitschke and Pastore, 2006), volatile aromatic compounds (Damasceno *et al*., 2003), and has even been considered for the production of probiotic beverages (Avancini *et al.*, 2007). However, the status of scientific knowledge in relation to CW microbial resources has been relatively superficial.

CW is a known veritable source of important microorganisms (Oyeleke and Oduwole, 2009; Ogbo, 2010; Akpomie *et al*., 2012), some of which may have industrial importance. The information on the microbial diversity of CW is scanty. Such information would enhance better utilization of the waste. It is possible that novel microorganisms of industrial importance may be identified especially if culture-independent molecular techniques are employed.

So far, studies on microorganisms associated with CW followed the classical culture isolation technique and subsequent identification based on morphological, physiological and biochemical portion of the true microbial population in a natural ecosystem (Elijah, 2013).

The increasing knowledge of gene sequences and the attendant development of new culture-independent molecular techniques are providing new and effective tools to analyse the diversity of microbial communities. In this study, molecular methods were employed, to characterize and identify bacteria species associated with CW, in order to reveal its true bacteria diversity

Materials and Methods

Sample collection and pre-treatment

CP waste from CP waste dumpsites and CWW from CWW discharge outlets were collected from major cassava processing centres in Abeokuta, Ogun State, Nigeria. Prior to DNA extraction, samples were pre-treated as follows: 5 g of CP waste was added to 10 ml of sterile physiological solution (0.9%, w/v NaCl) in a beaker containing glass beads and shaken with a vortex mixer (Vortex genie 2, Scientific Industries, USA) at room temperature (30 ± 2°C) for 15 min to dislodge microorganisms from the peels. The resulting mixture designated as pre-treated CP sample, was sieved using cheese cloth to remove debris. The filtrate from CP waste and the CWW, respectively, were centrifuged for 5 min at 4000 x g using a microcentrifuge (Spectrafuge 24D, Labnet International, USA). In each case, the pellet was discarded while the supernatant was centrifuged for 10 min at 10,000 x g to pellet cells. Cell pelleting was repeated until enough amount of cell was obtained. The pellet was washed in Tris-EDTA (TE) buffer, centrifuged for 10 min at 6000 x g and the supernatant was discarded. This step was repeated three times. The resulting cell pellet was used for bacterial DNA extraction.

Isolation of bacteria

characteristics which depend largely on the cultivation of the microbes and thus a great variation (or even errors) may likely occur. These methods are also time-consuming and laborious. Plate culturing techniques reveal only a little

For bacterial isolation, pre-treated CP sample was used while CWW was used directly. The pre-treated sample was allowed to settle and the supernatant was kept for use while the sediment was discarded. The supernatant as well as the CWW were diluted

serially to 10^{-8} and 10^{-5} respectively. One millilitre (1 ml) each was pour plated separately on nutrient agar and incubated at 37°C for 24 h for isolation of bacteria. Discrete representative colonies were picked from the plates and streaked out on nutrient agar to obtain pure cultures which were transferred to slant and stored in a refrigerator at 4°C.

Characterization and identification of bacterial species

Two molecular methods namely, total bacterial community DNA and isolates DNA sequence analysis were employed to characterize and identify bacteria species. The former method is a culture-independent method while the later is a culture-dependent method.

Total bacterial community DNA extraction

Total bacterial community DNA was extracted directly from CP waste and CWW respectively, using the DNeasy Blood and Tissue Extraction Kit (Qiagen, USA) according to the manufacturer's instructions. Cell pellet already washed in TE buffer was lysed in enzymatic lysis buffer (containing 2 mg/ml lysozyme, 25 Mm Tris HCl pH 8, 10 Mm EDTA, 25% sucrose) and incubated at 37°C for 30 min in an incubator (Uniscope SM9052, Surgifriend Medicals, England). Proteinase K and extraction buffer were added, mixed by vortexing and incubated at 56°C in a water-bath (Uniscope SM101 Shaking Water bath, Surgifriend Medicals, England) for 30 min. The DNA was precipitated with ethanol (96 – 100%, v/v) and transferred into the DNeasy Mini spin column for binding of DNA to the column, washed with two different 500 µl washing buffers and eluted with 200 µl elution buffer. The resulting DNA was stored at -20°C.

Bacterial isolate genomic DNA extraction

Genomic DNA extraction from bacterial isolates was also carried out using the DNeasy Blood and Tissue Extraction Kit (Qiagen, USA) following the protocol provided by the manufacturer. Overnight cultures grown in tryptone-soy broth (TSB) were centrifuged for 10 min at 5000 x g, to harvest cells. The pellet was washed 3 times in TE buffer. The procedure reported earlier for total bacterial community DNA extraction was followed subsequently to obtain the DNA.

Amplification of the 16S rRNA genes

The 16S rRNA gene from total bacterial community and genomic DNA respectively, was amplified by Polymerase Chain Reaction (PCR) using bacteria universal primers (27F – AGAGTTTGATCCTGGCTCAG and 1492R – GGTTACCTTGTTACGACTT). The PCR amplification was carried out in a Techne TC-412 Thermal Cycler (Model FTC41H2D, Bibby Scientific Ltd, UK) in a 50 µl reactions containing 25 µl of 2 X PCR Master Mix (Norgen Biotek, Canada), 1.5 µl of template DNA (0.5 µg), 1 µl of both forward and reverse primers (2.5 µM of each) and 21.5 µl of nuclease free water in a PCR tube added in that order. PCR was carried out at an initial denaturation step at 94°C for 2 min, followed by 30 cycles at 94°C for 30 sec, 52°C for 30 sec and 72°C for 2 min, and a final extension step at 72°C for 5 min. PCR products (amplicons) were separated by electrophoresis on a 1% agarose TAE gel containing ethidium bromide and visualized by UV transillumination (Foto/UV 15, Model 3-3017, Fotodyne, USA).

Cloning

Cloning was carried out using the the PCR TRAP Cloning System (GenHunter Corporation, USA), following the manufacturer's protocol. Amplicons from total bacterial community DNA were spliced into the PCR TRAP Cloning Vector using the T4 DNA ligase. Competent cells were transformed with the recombinant DNA and inoculated in Luria-Bertani (LB)-Tet agar (containing 20 µg/ml of tertacycline).

DNA sequencing and analysis

PCR products from the total community and genomic DNAs were sequenced with 518F

and 800R primers using ABI PRISM Big Dye Terminator cycle sequencer (Macrogen, USA). The gene sequences obtained were compared by aligning the result with the sequences in GenBank using the Basic Local Alignment Search Tool (BLAST) search program at the National Centre for Biotech Information (NCBI).

Results and Discussion

The 16S rRNA gene from total bacterial community DNA was successfully cloned into the PCR-TRAP Cloning Vector. Colonies of *E. coli* cells transformed with recombinant PCR-TRAP cloning vector carrying the 16S rRNA gene fragments of total bacteria community DNA from CP and CWW are shown in Fig. 1 and 2. Only positive transformants (*E. coli* cells carrying the 16S rRNA gene fragments from total bacteria community DNA) were able to grow on LB-Tet agar.

Microorganisms associated with cassava peel

Sixty two bacterial clones were detected and sequenced from the 16S rRNA gene library of CP. Identities of the sequenced clones are presented in Table 1. The sequences fell into 5 major lineages of the bacteria domain namely: *Bacillus–Bacillales* (30 clones), *Bacillus-Lactobacillales* (22 clones), Gamma-proteobacteria (5 clones), *Acinetobacteria - Actinomycetales* (2 clones) and uncultured bacteria (3 clones). However, 31 bacterial isolates were identified in CP as shown in Table 2.

Based on the 16S rRNA gene sequence analysis, the bacterial isolates were grouped into *Bacillus – Bacillales* (17 isolates), *Bacillus-Lactobacillales* (11 isolates), Gamma-proteobacteria (2 isolates), *Acinetobacteria -Actinomycetales* (1 isolate). In all, a total of 24 bacterial species, belonging to *Bacillus–Bacillales* (7 species), *Bacillus-Lactobacillales* (11 species), Gamma-proteobacteria (3 species), *Acinetobacteria-Actinomycetales* (1 species) and unidentified bacteria (2 species), were detected in CP. The result showed that CP waste is characterized by an array of bacterial dominated by *Bacillus licheniformis* and *B. Substilis* each having a relative abundance of 11.3%. Previous studies (Oyeleke and Oduwole, 2009; Akpomie *et al.,* 2012) have also reported the dominance of *Bacillus* species in CP. The capacity of most *Bacillus* strains to produce and secrete large quantities of extracellular enzymes (Schallmey *et al.*, 2004), might have enabled them to breakdown and utilize of CP thus enhancing their growth and proliferation over other strains. Amoa-Awua *et al.* (1997) demonstrated the ability of some *Bacillus* species to break down cassava tissue. *Bacillus* species have also been reported to biodegrade cyanide to non-toxic end products by using cyanide as a sole nitrogen source under aerobic and/or anaerobic environment (Sirianuntapiboon and Chuamkaew, 2007). This could also be an advantage to its proliferation in the cyanide-rich CP.

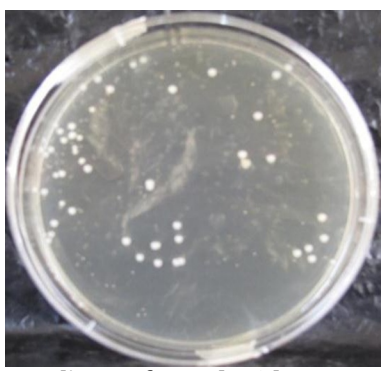

Fig. 1: *E. coli* transformed with 16S rRNA gene fragments from bacteria community DNA from cassava peel

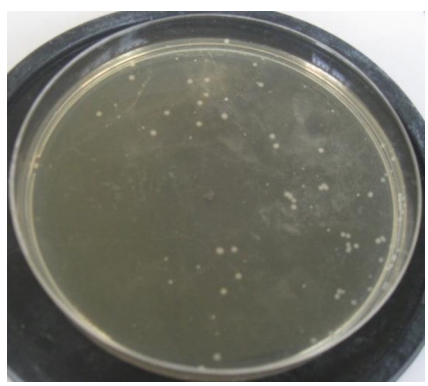

Fig. 2: *E. coli* transformed with 16S rRNA gene fragments from bacteria community DNA from cassava wastewater

Table 1: Distribution of bacteria identified in the 16S rRNA gene clone library of cassava waste bacterial community DNA

Clone identity/bacteria domain	Accession number	% identity	No of clones (relative abundance)	
			P	W
Bacillus – Bacillales				
Bacillus substilis strain 22	FJ435215.1	100	7(11.3%)	2(3.7%)
Bacillus coagulans strain LCR24	FJ976533.1	98	3(4.8%)	-
Bacillus pumilus strain PSL-J7-2	HQ218986.1	99	5(8.1%)	1(1.9%)
Bacillus licheniformis strain S1	GQ141054.1	100	7(11.3%)	2(3.7%)
Bacillus circulans	JN644554.1	100	2(3.2%)	-
Bacillus cereus	HQ259952.1	97	4(6.4%)	1(1.9%)
Staphylococcus xylosus	JN644524.1	99	2(3.2%)	-
Staphylococcus aureus	U02910.1	100	-	3(5.6%)
Bacillus-Lactobacillales				
Lactobacillus plantarum	AF095564.1	100	4(6.4%)	5(9.3%)
Lactobacillus fermentum	AF477498.1	99	4(6.4%)	6(11.1%)
Lactobacillus pentosus	AF375905.1	100	1(1.6%)	3(5.6%)
Lactobacillus manihotivorans	AB289202.1	95	2(3.2%)	2(3.7))
Lactobacillus delbrueckii	AY675257.1	99	-	1(1.9%)
Lactobacillus brevis	JX398133.1	100	2(3.2%)	1(1.9%)
Lactobacillus perolens	NR_029360.1	100	-	1(1.9%)
Lactobacillus sanfrancisco	X76331.1	97	-	2(3.7%)
Lactobacillus crispatus	AB008206.1	99	-	2(3.7%)
Leuconostoc mesenteroides	JX423551.1	98	3(4.8%)	4(7.4%)
Leuconostoc citreum	NR_041727.1	100	-	2(3.7%)
Leuconostoc cremoris	M23034.1	100	1(1.6%)	2(3.7%)
Leuconostoc lactic	D31668.1	100	-	2(3.7%)
Leuconostoc fallax	AB362604.1	100	1(1.6%)	2(3.7%)
Weisella confusa	EU807754.1	99	-	3(5.6%)
Pediococcus pentosaceus	FJ844959.1	100	1(1.6%)	1(1.9%)
Enterococcus faecalis	FJ804073.1	100	2(3.2%)	-
Enterococcus saccharolyticus	AB681191.1	100	1(1.6%)	-
Gamma proteobacteria				
Pantoea agglomerans	JX257179.1	99	1(1.6%)	1(1.9%)
Enterobacter aerogenes	AJ251468.1	100	2(3.2%)	-
Propionibacterium theonii	AY883045.1	100	-	1(1.9%)
Azotobacter vinelandii	AY336565.1	98	2(3.2%)	-
Acinetobacteria Actinomycetales				
Corynebacterium glutamicum	DQ173748.1	99	2(3.2%)	1(1.9%)
Beta proteobacteria				
Alcaligenes faecalis	NR_043445.1	100	-	2(3.7%)
Uncultured bacteria				
Uncultured bacterium clone ncd1462c07c1	JF126050.1	99	1(1.6%)	1(1.9%)
Uncultured compost bacteria clone PS2630	FN667396.1	100	2(3.2%)	-

P = cassava peel, W = cassava wastewater. Sequences < 96% identity with the closest known relative in databases is considered a novel species (Miambi *et al*., 2003).

Table 2: Distribution of bacteria identified in the 16S rRNA gene sequence analysis of cassava waste bac-teria isolates' DNA

Isolate identity/Bacteria domain	Accession number	% identity	No of isolates (relative abundance)	
			P	W
Bacillus – Bacillales				
Bacillus substilis	FJ435215.1	100	6(19.4%)	-
Bacillus coagulans	FJ976533.1	99	1(3.2%)	-
Bacillus pumilus	HQ218986.1	94	2(6.4%)	-
Bacillus licheniformis	GQ141054.1	98	4(12.9%)	-
Bacillus circulans	JN644554.1	99	1(3.2%)	-
Bacillus cereus	HQ259952.1	99	2(6.4%)	2(9.5%)
Staphylococcus xylosus	JN644524.1	98	1(3.2%)	-
Bacillus-Lactobacillales				
Lactobacillus plantarum	AF095564.1	100	2(6.4%)	3(14.3%)
Lactobacillus fermentum	AF477498.1	99	1(3.2%)	3(14.3%)
Lactobacillus pentosus	AF375905.1	100	-	1(4.8%)
Lactobacillus delbrueckii	AY675257.1	99	1(3.2%)	2(9.5%)
Lactobacillus brevis	JX398133.1	100	1(3.2%)	1(4.8%)
Lactobacillus perolens	NR_029360.1	100	-	1(4.8%)
Leuconostoc mesenteriodes	JX423551.1	98	2(6.4%)	3(14.3%)
Leuconostoc cremoris	M23034.1	100	1(3.2%)	1(4.8%)
Leuconostoc fallax	AB362604.1	100	-	2(9.5%)
Weisella confusa	EU807754.1	99	-	1(4.8%)
Pediococcus pentosaceus	FJ844959.1	100	1(3.2%)	1(4.8%)
Enterococcus faecalis	FJ804073.1	100	1(3.2%)	-
Enterococcus saccharolyticus	AB681191.1	100	1(3.2%)	-
Gamma proteobacteria				
Azotobacter vinelandii	AY336565.1	98	2(6.4%)	-
Acinetobacteria Actinomycetales				
Corynebacterium glutamicum	DQ173748.1	99	1(3.2%)	-

P = cassava peel, W = cassava wastewater. Sequences < 96% identity with the closest known relative in databases is considered a novel species (Miambi *et al*., 2003).

Furthermore, this study revealed the presence of bacteria species such as *Staphylococcus xylosus, Lactobacillus manihotivorans, Lactobacillus pentosus, Leuconostoc fallax, Pediococcus pentosaceus, Propionibacterium theonii, Enterococcus saccharolyticus, Azotobacter vinelandii, Pantoea agglomerans, Enterococcus faecalis, Enterobacter aerogenes* and unidentified bacteria (similar to uncultured bacterium clone ncd1462c07c1 and uncultured compost bacteria clone PS2630), hitherto not detected in CP. This further confirmed the limitation of culture and conventional identification methods (used by previous researchers) in understanding the microbial diversity of CP. Phylogenetic analysis (result not presented) suggests that the unidentified bacteria having high similarity to uncultured bacterium clone BCP23 and uncultured compost bacterium clone BCP24 are closely related to *Bacillus cereus*.

Some of the species identified in this study have been implicated during the processing of cassava into various products. For instance, Ampe *et al*. (2001)

reported the presence of *Enterococcus saccharolyticus* in spontaneous fermentation of cassava starch in Colombia. The author also detected the presence of *Lactobacillus manihotivorans* by hybridization of 16S rRNA gene fragments with phylogenetic probes. Similarly, Oguntoyinbo (2007) isolated *Leuconostoc* and *Weisella* species from cassava fermentation for gari production, while Oguntoyinbo and Dodd (2010) reported the presence of *L. pentosus* at the initial part of the fermentation. The association of lactic acid bacteria (LAB) with the fermentation of plant materials has been repeatedly reported (Stiles and Holzapfel, 1997). However, *Azotobacter vinelandii*, the two unidentified bacteria similar to an uncultured bacterium clone ncd1462c07c1, and an uncultured compost bacterium clone PS2630 respectively, have not been reported previously in cassava fermentation, suggesting they could possibly constitute novel species.

Azotobactor vinelandii is an aerobic nitrogen-fixing Gram-negative bacterium found in soils, with the capacity to fix nitrogen gas (N_2) to compound of ammonium (NH_4^+) through the action of nitrogenase (Marín *et al*., 2001). Kaewkannetra *et al*. (2009) reported the ability of *A. vinelandii* to grow and remove cyanide in CWW. The mechanism of cyanide reduction is thought to be associated with the N_2-fixing enzymes. The enzymes, rhodaneses and nitrogenise, elaborated by these strains have been reported to be involved in cyanide detoxification (Fisher *et al*., 2000). This suggests the potentials of *Azotobacter vinelandii* as a possible candidate for cyanide detoxification in CP before use as animal feed. CP normally have higher concentration of cyanogenic glucosides than the parenchyma (pulp) (Ubalua, 2007), thus making the peel unsuitable for animal feed except after proper processing.

Microorganisms associated with cassava wastewater

The distribution of bacteria identified in the 16S rRNA clone library of CWW from bacterial community DNA is also presented in Table 1. The result showed that 54 bacterial clones were detected in the 16S rRNA clone library of CWW. These included *Bacillus–Bacillales* (8 clones), *Bacillus-Lactobacillales* (40 clones), Gamma-proteobacteria (2 clones), *Acinetobacteria-Actinomycetales* (1 clone), Beta proteobacteria (2 clones) and unidentified bacteria (1 clone). However, 21 bacterial isolates were obtained from CWW. Analysis of the 16S rRNA gene sequence of the bacterial isolates showed that they belong to *Bacillus–Bacillales* (2 isolates) and *Bacillus-Lactobacillales* (19 isolates), as presented in Table 2. From the two identification methods, a total of 26 bacterial species were detected including *Bacillus–Bacillales* (5 species), *Bacillus-Lactobacillales* (16 species), Gamma-proteobacteria (2 species), *Acinetobacteria-Actinomycetales* (1 species), Beta proteobacteria (1 species) and an unidentified bacteria (1 species). *Lactobacillus fermentum* (11.1%) was the dominant species closely, followed by *L. plantarum* (10.7%).

Earlier findings have shown that cassava starch fermentation wastewater is composed mainly of LAB with predominance of the genera *Lactobacillus* (Avancini *et al*., 2007). Consequently, cassava starch fermentation wastewater, due to its rich probiotic components (lactic acid bacteria and yeasts) with the status, generally recognized as safe (GRAS), has been considered for possible production of probiotic beverage (Avancini *et al*., 2007). Generally, cassava fermentation is a typical lactic acid fermentation in which varieties of microorganisms are present at the beginning, but as the fermentation progresses, these microorganisms are gradually overgrown by lactic acid bacteria, predominated by the genera *Lactobacillus* (Lacerda *et al*., 2005). Thus LAB which are known to be responsible for the production of organic acids, are the prevalent microorganisms associated with the spontaneous fermentation of cassava starch (ben-Omar and Ampe, 2000). Ampe *et al*. (2001) suggested that the succession is determined by the sensitivities of microorganisms to the very acidic conditions that develop during the process.

The diverse strains of LAB identified in this work have been reported to have different desirable functionalities during fermentation of vegetal starch (Miambi *et al*., 2003). Most LAB produce linamarase which enables them to tolerate the high cyanide content of CWW, containing as high as 200 mg l^{-1} depending on the cyanoglycoside content of the cassava varieties (Siller and Winter, 1998). Microorganisms with linamarase activities are desirable as they can be used to detoxify cyanogenic glycosides containing food and feed materials. Adamafio *et al*. (2010) isolated *Lactobacillus* spp. in fermented cassava pulp juice which was capable of reducing the levels of cyanogenic glycosides in CP to non-toxic levels and also improving the nutritional value of the peels by increasing the protein content of the peels appreciably. This suggests the complementary role CP and CWW could play as CWW which is usually discarded by cassava processing industries, can be fermented and effectively utilized to achieve remediation of CP, another waste product.

Prevoius studies in CWW could not detect the presence of *Lactobacillus manihotivorans, L. confusa, L. sanfrancisco, L. crispatus, L. cellobiosus, Leuconostoc citreum, L. lactic, Pantoea agglomerans, Propionibacterium theonii* and an unidentified bacterium similar to an uncultured bacterium clone ncd1462c07c1, detected in the present study. This may be due, largely, to the inability of the traditional cultural identification methods employed in those studies, to detect all organisms present, especially the viable but non-culturable (VBNC) ones. Some of these species have been reported to be involved during the processing of cassava into various products, especially using molecular tools. Among these,

Pantoea agglomerans has demonstrated its effiicacy in controlling important postharvest diseases of apples and pears (Nunes *et al*., 2002) and of citrus fruit (Usall *et al*., 2008). It would therefore be of interest to investigate the potential of *Pantoea agglomerans* from CW as a biological control agent (BCA) in fruits and vegetable preservation.

A comparison of bacterial species identified by total bacterial community DNA sequence analysis with the ones identified by isolates DNA sequence analysis showed that more bacterial species were detected by the former method than by the later. This result was expected due to the inherent bias associated with traditional cultivation approaches.

Viable but non-culturable ('VBNC') species which could not be isolated and thus not detected by isolate DNA sequence analysis were detected by the culture-independent total bacterial community DNA sequence analysis.

In principle, all the species that were cultured should have been found in the clone library. However, this was not the case as few isolates were not found in the clone library, demonstrating that culture-dependent and culture-independent methods are complementary. The use of nutrient agar, as the only culture medium for bacteria, in this study, may have limited the growth of some lactic acid bacteria (LAB). Nevertheless, the culture-independent approach also adopted addressed this disadvantage, thereby missing out no bacterial species in the clone library. It is generally believed that uncultured organisms comprise the vast majority of the microbial world (Head *et al*., 1998).

Conclusion

This study has shown that CW is an important microbial resource, considering its rich bacterial diversity. The results indicated that *Bacillus licheniformis* and *Bacillus substilis* are the dominant bacterial species in CP waste, while *Lactobacillus fermentum* and *Lactobacillus plantarum* are the dominant bacterial species in CWW. The use of molecular identification methods has further revealed novel bacteria species hitherto not reported in CW, thus demonstrating the importance and the need to adopt this new approach in studying microbial diversity in food and related samples.

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