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Isolation and identification of cyanide tolerant bacteria from cassava effluent

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Abstract
Cyanide is a group of compounds which contains a C≡N group: one atom of carbon linked with one atom of nitrogen by three molecular bounds. The cyanide component of cassava mill effluent is highly toxic to man and its environment. Presence of cyanide resulting from the discharge of untreated CME into soil could prevent oxidation/reduction process in non-resistant microbes, thereby leading to decline in productivity probably due to the effect on soil microorganisms. One mill of the soil sample was introduced into a sterile petri dish and Nutrient Agar was also introduced inside the petri dish and placed in an incubator for 24 hours for the bacterial count. The microbial isolates were *Proteus mirabilis*, *Proteus vulgaris*, *Staphylococcus saprophyticus*, *Bacillus* spp, *Klebsiella*, *Streptococcus*, *Pseudomonas* spp, *Acinetobacter*, *Burkhoderia*, *Proteus mirabilis*, *Proteus vulgaris*, *Pseudomonas* spp *Staphylococcus saprophyticus*, *Bacillus* spp, *Klebsiella*, *Streptococcus*, The microbial isolates gotten from sample A and B tends to survive in high concentration of cyanide content in cassava effluents and are good in detoxifying the cyanide content which seems to be a pollutant to the environment and harmful substance to the plants.

Keywords: Cyanide, toxic, cassava, microbial

Introduction
Cyanide is a group of compounds which contains a C≡N group: one atom of carbon linked with one atom of nitrogen by three molecular bounds, Moradkhani *et al.* (2018); Nwokoro and UjuDibua (2014); Razanamahandry *et al.* (2017). In the environment, cyanides can be found in many different forms (Kuyucak and Akcil, 2013; Mirizadeh *et al.*, 2014) [11, 9]. It is also defined as a toxic nitrogen compound produced by living organisms comprising algae, bacteria, fungi, and plants as part of a defense mechanism against predation (Maniyam *et al.*, 2013) [9]. Nevertheless, these natural sources of cyanide are inconsequential in pollution of the environment in comparison to cyanide production through anthropogenic activities (Zohre *et al.*, 2017) [9]. Cyanide is lethal to humans and animals (Parker-Cote *et al.*, 2018; Tiong *et al.*, 2015; Uzoije *et al.*, 2011) and thus wastewater containing cyanide poses a threat to aquatic organisms and terrestrial organisms that utilize water on the mainland (Mekuto *et al.*, 2013). Thus, the consumption of an inadequately processed cassava product for prolonged periods may result in chronic toxicity. However, certain varieties contained large amount of cyanogenic glycosides (linamarin and lotaustralin) which can hydrolysed to hydrocyanic acid (HCN) by their endogenous enzyme (linamarase) when the plant tissue is damaged during harvesting, processing or other mechanical processing (Oboh and Akindahunsi, 2003b) [7]. It is native to South America and is extensively cultivated in the tropical and subtropical regions of the world for its edible starchy tuberous root and are harvested between 7 to 13 mouths based on the cultivars planted (Cooke, 1985); Taye, 1994). The tubers are quite rich in carbohydrates (85-90%) with very small amount of protein (1.3%) in addition to cyanogenic gloucoside (Nwabueze and Odunsi, 2007; Oyewole and Afolani, 2001). As a major source of carbohydrate in the world with Africa being the largest centre of production (Claude and Denis, 1990). Annual cassava production in Africa is about 84 million tones, with Nigeria being the highest with 30 million tones. Okafor (1998) and Oboh and Akindahunsi (2003b) [7] separately reported the bioconversion of agricultural wastes of microbial isolates of which Cassava waste water is not an exception. The growth and survival of these microbial isolates may not be unconnected with the fact that the waste water contained substances that can be utilized by the isolates. Uzochukwu *et al.* (2001) and Oboh (2005) had earlier reported the composition cassava waste water. These microbial isolates may probably have originated from soil, water and materials used during

the processing of cassava, while the variations of the isolates may be due to the handing process and the prevailing environmental conditions. Therefore, the isolates can be said to be transient microorganisms surviving only in the absence or low cyanide and/ or other inhibitory substances in cassava (Olowoyo *et al*., 2001). FAO (2008) found that, the total bacteria (Lactobacilus planetarium, *Pseudomonas aeruginosa*, *Bacillus* spend *vitro* spp). Obtained from the contaminated soil with cassava effluent wasmore in the soil than that inthe soil without contaminant. According to Nwankwo *et al* (2005). Organisms isolated during the fermentation of cassava tubers as practicefor "fufu" production included *Bacillus* subtilis, *Pseudomonas* alealigenes, Lacto *Bacillus* planetarium, Leuconostic mensenteriodes and *Pseudomonas aeruginosa*. Oboh (2005) identified two important wastes that are generated during the processing of cassava tubers to include cassava peels and the liquid squeezed out of the mash. The bioconversion of the cassava wastes have been documented (Antia and Mbongo, 1994, Oboh, 2005). The (waste water) contains heavy loads of microorganisms, lactic acid, lysine (from L. coryneformis), and amylase (from *L. delbruckii*) capable of hydrolyzing the glycosides (Raimbault, 1998; Akindahunsi *et al*., 1999) [7]. 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. Cyanoglucosides are secondary metabolites produced by several plant species used in animal and human diets, such as: apple, bamboo shoot, cassava, cherry, lima bean, maize, oat, peach, papaya, sorghum and wheat. These compounds are dispersed throughout the plant organs, mostly in nonedible parts, but may become concentrated in edible roots and leaves, as in the case of cassava. Besides large quantities of soil, discharged wastewater contains a number of contaminating substances. The objective of this research is to isolate and identify bacteria tolerant to cyanide in cassava effluent.

Methodology

Sample Collection Technique

Soil samples were collected using the transect survey (straight line) method. Soil samples (polluted) were collected at different points though, in the same town at depth of 10 to 15 centimeters using soil Auger. The samples were collected into different polythene bags and transported to Federal polytechnic ileoluji Biology laboratory for microbial analysis.

Sterilization of Equipment and Materials

Glass wares such as test tubes, Petri dishes were sterilized by standard methods as described by Cheesborough (2000) and Pelczar *et al*. (1986). Forceps, spatulas and slides were also sterilized by hot air oven at a temperature of 105oC for 2 hours. Wire loops were sterilized by flaming to red hot.

Preparation of the Samples

The samples were used fresh for the microbial analysis to avoid loss of some members of microbial flora also, to avoid contamination. One gram (1g) of each sample was measured and dispensed in 10ml test tube of sterile distilled water. An aliquot of each sample was used for microbial analysis.

Preparation of media

Preparation of Nutrient Agar (NA)

This was prepared according to the manufacturer's instructions. Twenty eight grams (28g) of dehydrated Nutrient Agar base medium was dissolved in about 1000ml of distilled water. The mixture was heated in a water bath until the Agar melted. It was made up to 1 litre and its PH checked to conform to the standard (7.2 to 7.6). The prepared medium was used for plate preparation. Before sterilization of the medium, a part of it was dispensed in 15ml volume into McCartney bottles. These and the other remaining portion were sterilized in an autoclave at 121oC and 15Psi for 15minutes. After autoclave, the McCartney bottles with sterilized medium were arranged in racks to cool and gel in a slanted position to form sterile Agar slants used for sub cultures. The bulk of the medium was allowed to cool to 45oC before it was aseptically poured into sterile Petri dishes to form agar gel used for bacterial propagation.

Determination of Bacteria counts

Serial dilution of Cassava Effluent Samples

Five – fold serial dilution of the cassava effluent sample was prepared as defined by cheesbrough (2006). Ten (10) mill of distilled water was measured in a test tube and one (1) gram of the sample was measured inside the ten (10) mill of distilled water. Thereafter, one (1) mill of the cassava effluent sample was transferred to the next test tube until the fifth test tube which gave the dilution factor of 1×10-5.

Inoculation and Enumeration

Aliquot 1ml of five - fold serial dilution of the cassava effluent samples was inoculated into the Nutrient agar plates applying pour plate method (Cheesbrough 2006). The Inoculated petri dishes were incubated for 24 hours in an incubator for the enumeration of the total bacterial counts.

Preparation of pure cultures of Isolates

After incubation and establishment of growth, Nutrient Agar plate were observed for discrete colonies. Inoculums from such discrete colonies were aseptically taken and sub cultured into sterile nutrient agar slants. Nutrient Agar slants were incubated at 37°C for 24 hours. This pure culture of bacteria obtained were used for identification.

Identification of bacterial Isolates

Each bacteria isolates to be identified was subjected to systematic step by step analysis and examination as described by Cheesbrough (2000). At the end, the characteristics of individual isolates were matched against those in Buchanan and Gibbons (1974).

Results

Table 1: Isolates identified

Samples	Isolates identified.
Sample A	*Proteus mirabilis*, *Proteus vulgaris*, *Staphylococcus saprophyticus*, *Bacillus* spp, *Klebsiella*, *Streptococcus*, *Pseudomonas* spp
Sample B	*Acinetobacter*, *Burkhoderia Proteus mirabilis*, *Proteus vulgaris*, *Pseudomonas* spp *Staphylococcus saprophyticus*, *Bacillus* spp, *Klebsiella*, *Streptococcus*,

The results obtained herein revealed the isolation of

microbial strains with high ability of tolerating cyanide content from cassava effluent and also biodegrading the cyanide contents. *Bacillus* spp, *Pseudomonans* spp had the maximum cyanide utilizing ability (turbidity) of all the microbial isolates screened. This further attested that these microorganisms have the capabilities to survive favourably in cassava mill effluent and alter them to non-toxic products.

Sample A has *Proteus mirabilis*, *Proteus vulgaris*, *Staphylococcus saprophyticus*, *Bacillus* spp, *Klebsiella*, *Streptococcus*, *Pseudomonas* spp as the microbial isolates while Sample B has *Acinetobacter*, *Burkhoderia Proteus mirabilis*, *Proteus vulgaris*, *Pseudomonas* spp *Staphylococcus saprophyticus*, *Bacillus* spp, *Klebsiella*, *Streptococcus* as the microbial isolates. Looking at the result from Sample A and sample B, they are almost the same except *Acinetobacter* which is present in sample B ans absent from sample A which shows that it has the high tendency of surviving in cassava mill effluent with very high cyanide.

Conclusions and Recommendations

The microbial isolates gotten from sample A and B tends to survive in high concentration of cyanide content in cassava effluents and are good in detoxifying the cyanide content which seems to be a pollutant to the environment and harmful substance to the plants.

Recommendation

This work shows microorganisms which tends to survive in cassava mill effluent with high cyanide, it is hereby recommended that further research should be carried out on how to introduce these bacteria isolates which can serves as a control measures that can convert cyanide to non-toxic substance.

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