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Antagonistic Activity of Mycoflora Associated with Cassava Whey Obtained from Cassava Mills in Benin City, Nigeria

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ABSTRACT: Whey (cassava wastewater) is an industrial residue obtained from the processing of cassava into various fermented products such as *garri, fufu* and *lafun*. Cassava whey mycoflora and their antagonistic activity against *Escherichia coli* were determined in this report. The associated fungi were enumerated and isolated by standard microbiological methods. The pH value of cassava whey samples was determined using an electrode pH meter while titratable acidity was by acid-base titration. Antagonistic effects of the fungal isolates against previously identified diarrheagenic *E. coli* strain 838 and 848 was determined using the agar well diffusion technique with the culture supernatant of each fungus. The highest fungal counts were recorded in factory B (7.80×10^7 cfu/ml) while factory D had the least count of 12.90×10^7cfu/ml. The fungal isolates identified include; *Saccharomyces cerevisiae, Aspergillus niger, Aspergillus flavus*, *Rhizopus*, *Fusarium* and *Mucor* species with *Saccharomyces cerevisiae* having the highest frequency of occurrence (61.10%) in all the samples. The pH values and titratable acidity of the fufu whey samples ranged from 3.75±0.33 to 4.54±0.08 and 3.90±0.14 to 6.06±0.33% respectively. The zone of inhibition of the fungal isolates against *E. coli* strain 838 ranged from 18.50 ± 2.12mm (*Rhizopus* sp.) to 25.00 ± 0.00 mm (*Saccharomyces cerevisiae*) while the zone of inhibition against *E. coli* strain 848 ranged from 26.00 ± 1.14 mm (*Mucor* sp.) to 38.00 ± 1.14 mm (*Saccharomyces cerevisiae*). Cassava whey can be converted into economic value by propagation of yeast biomass for the production of value added products.

Keywords: Cassava whey, antagonistic activity, fungi

Introduction

Cassava (*Manihot esculenta*) is a major staple food in the world, especially in many developing countries in Africa. The tubers are rich in carbohydrates (85-90%) with a very small amount of protein (1.30 %) in addition to cyanogenic glucosides (Nwabueze and Odunsi, 2007; Eze and Onyilide, 2015). Nigeria is the largest cassava producing nation in the world accounting for over 20% of global production. Cassava is processed into *lafun*, *fufu* and *garri*, however its processing in Nigeria is carried out by small holders who occupy 80 % of the sector (Izah, 2018a). Microorganisms involved in the processing of cassava into fermented products include *Leuconostoc* sp., *Lactobacillus* sp., *Corynebacterium* sp., *Candida tropicalis*, *Streptococcus* sp. and *Geotrichum candidum* (Oyewole and Isah, 2012). Cassava has a great potential to be used in Africa. This is because cassava flour and starch can perform most of all the functions where rice, wheat and maize starch are currently used (Avwioroko and Tonukari, 2014). Processing of cassava generates huge amounts of wastes which include peels, sievate, stumps and whey. The two important wastes generated during processing of cassava tubers include cassava peels and the liquid squeezed out of the mash (Oboh, 2005). Cassava whey is the liquid pressed out of

the tuber after it has been crushed mechanically (Anusi *et al*., 2018). The discharge of agricultural by-products such as cassava waste from processing activities is a growing concern in Nigeria (Eze and Onyilide, 2015). The disposal of cassava waste causes environmental problems due to its high organic load; however it is a rich source of carbohydrate, water and minerals that support the growth of microbes including fungi (John and John, 2015; Avwioroko and Tonukari, 2014). Previous studies have shown that cassava waste water could be utilised for the production of yeast biomass, enzymes, biofertilizers, organic acids, biosurfactants, butanol and volatile aromatic compounds (Oshoma *et al*., 2010; Izah, 2018b; Ogbo, 2010; John and John, 2015). It has even been considered for the production of probiotic beverages (Avancini *et al*., 2007). Efficient utilization of cassava waste for value addition depends on proper understanding of its true microbial diversity. Cassava waste is a veritable source of microorganisms of industrial importance; however, information in the microbial diversity of cassava waste is scanty (Elijah and Asamudo, 2016). The aim of this research was to isolate and identify fungi associated with cassava whey and evaluate their antagonistic activity against pathogenic *Escherichia coli* strains.

Materials and Methods

Sample collection: Cassava whey was obtained from five different cassava processing factories in Benin City, Edo State, Nigeria. The samples were collected in sterile bottles and transported to the Department of Microbiology Laboratory, University of Benin, Benin City, Nigeria, for analyses.

Isolation and identification of fungi: The fungi were isolated by serial dilution and the pour plate method. Collected whey samples were serially diluted and 0.10 ml (dilution 10^{-5}) of samples was inoculated on potato dextrose agar (PDA) using the pour plate method. The inoculated plates were incubated at 28 ±2ºC for 3-5 days. Colonies that developed were counted and recorded as colony forming unit per millilitre (cfu/ml). Pure cultures were obtained by sub culturing and identified by morphological and microscopic observation under x40 magnification (Barnett and Hunter, 1972).

Determination of pH value: The pH value of each sample was determined using the Extech Instrument after standardization with appropriate buffers. The electrode sensor of the pH meter was inserted directly into 20 ml sample in a clean 50 ml glass beaker. The value was read and recorded.

Determination of total titratable acidity: This was carried out according to the method of Arotupin (2007). Twenty-five millilitre (25.00 ml) of the samples with 3 drops of 1% phenolphthalein indicator was titrated against 0.1M NaOH. The end point was recorded when the pink colour was noted. The total titratable acidity was calculated as lactic acid; volume of 0.1M NaOH x factor x100 over volume of weight of sample used in titration (ml).

Antagonistic e'fficacy of fungal isolates against E. coli: The antibacterial activity of fungal isolates was evaluated using the agar well diffusion method. The test antagonists were prepared by inoculating each isolates aseptically into potato dextrose broth and incubating at 28±2ºC for 48 hr. Cells were removed by centrifuging at 4000 rpm for 15 min. Cultures of *E. coli* stain E838 and E848 were obtained from the stock culture collection of the University of Benin Teaching Hospital (UBTH), Benin City, Edo State, Nigeria. The culture were grown on tryptic soy broth at 37ºC for 18-24 hr. A 0.10 ml aliquot of test isolate was spread on Mueller-Hinton agar. Wells of 5 mm diameter were then bored in the plate using a sterile cork borer and 100 uL of supernatant extract of fungal isolate were infused into the well and incubated at 37ºC for 24 hr. The diameter of the zone of inhibition was measured in mm.

Results

The total fungal counts of cassava whey from each factory are presented in Table 1. Factory D had the least fungal count (7.80×10^7 cfu/ml) while factory B had the highest fungal count (12.90×10^7 cfu/ml).

Table 1: Total fungal count of cassava (fufu) whey samples from cassava mills in Benin City

Cassava Mills	Fungal Count (cfu/ml)
A	9.60×10^7
B	12.90×10^7
C	10.80×10^7
D	7.80×10^7
E	11.60×10^7

The distribution of the fungi identified using cultural and morphological characteristics are presented in Table 2. The result showed that six fungal species were isolated. They include *Aspergillus* niger (9.10 %), *Aspergillus flavus* (5.70 %), *Saccharomyces cerevisiae* (61.10 %), *Rhizopus* sp. (8.92 %), *Mucor mucedo* (7.02 %) and *Fusarium* sp. (8.16 %), the dominant species was *Saccharomyces cerevisiae*.

Table 2: Percentage occurrence of fungal iIsolates in cassava whey samples from cassava mills in Benin City

Fungal Isolates	Percentage Occurrence (%)
Saccharomyces cerevisiae	**61.10**
Rhizopus sp.	**8.92**
Aspergillus flavus	**5.70**
Aspergillus niger	**9.10**
Fusarium sp.	**8.16**
Mucor sp.	**7.02**

The values of pH and total titratable acidity are presented in Table 3. The pH values ranged from 3.75 ± 0.33 (Factory A) to 4.54 ± 0.08 (Factory E) while the total titratable acidity ranged from 3.90 ± 0.14% (Factory E) to 6.06±0.33% (Factory A).

Table 3: Physicochemical quality of cassava (fufu) whey samples from cassava mills in Benin City

Cassava mills	pH	Titratable acidity (g/100ml)
A	3.75±0.33	6.06±0.33
B	4.20±0.50	4.90±1.00
C	4.30±1.23	4.20±1.54
D	4.45±0.07	4.50±0.50
E	4.54±0.08	3.90±0.14

All fungal isolates showed variable antibacterial activities against *E. coli* strain 838 and 848 (Table 4). *Saccharomyces cerevisiae* showed high antibacterial activity against *E. coli* strain E838 (25.00 ± 0.00) and E848 (38.00 ± 1.41mm) while *Rhizopus* sp. showed the lowest antibacterial activity against *E. coli* strain 838 (18.00 ± 2.12mm).

Table 4: Antagonistic activity of fungal isolates from cassava (fufu) whey against diarrheagenic *E. coli*

Organisms	Zone of clearance (mm) E838	Zone of clearance (mm)E848
Saccharomyces cerevisiae	25.00±0.00	38.00±1.41
Rhizopus sp.	18.50±2.12	32.5±0.71
Aspergillus flavus	22.00±1.40	29.00±1.41
Aspergillus niger	19.00±2.83	30.50±3.54
Fusarium sp.	20.50±2.12	36.00±1.41
Mucor sp.	20.50±2.13	26.00±1.14

Discussion

Cassava whey is a rich source of nutrients that supports the growth of microorganisms. Uzochukwu *et al*. (2001) reported that cassava waste water contains fermentable sugars, starch and cellulose. Oboh (2005) also documented that it contains essential elements. This may account for the high fungal count, growth and survival of the diverse fungal species isolated from cassava whey. However, fungi may also have been introduced through cassava handlers, water and equipment used during the processing of cassava.

The pH values of cassava whey sampled in this study were all acidic ranging from 3.75 ± 0.33 to 4.54 ± 0.08. Izah (2018a) reported that there is increased microbial population of bacteria and fungi and a decline in pH during fermentation of cassava tuber for fufu production. Arotupin (2007) and Uzochukwu *et al*. (2001) also reported pH range of 3.55 to 4.20 which is in agreement with this finding. However, Anusi *et al*. (2018) reported the pH of cassava whey to be 5.82, which is slightly acidic. The total titratable acidity values observed in this study were lower than that reported by Arotupin (2007). The difference may be as a result of the amount of other organic acids in the whey. It has been reported that the effluents generated during the processing of cassava into garri is acidic (Izah, 2018a). Oti (2002) revealed that the cyanide in cassava forms an acidic complex called hydrocyanic acid which could attribute to the acidic nature of cassava whey.

Different reports have noted the presence of *Aspergillus niger, A. flavus, A. fumigatus, Geotrichum candidum, Rhizopus* sp., *Saccharomyces* spp. and *Candida* spp. from cassava waste water (Elijah and Asamudo, 2016; Arotupin, 2007). Some of the fungal species isolated have been reported to be associated with cassava fermentation for various products.

The findings of this study showed that *Saccharomyces cerevisiae* was the dominant fungal species with percentage occurrence of 61.10 %. Previous studies by Elijah and Asamudo (2016) who reported 47 % occurrence of yeasts, and Arotupin (2007) have also reported the presence of different species of yeasts including *Saccharomyces cerevisiae, S. exguus, Candida utilis, Geotrichum candidum* and *Saccharomyces* spp.

Yeasts have been identified as the second predominant microorganism involved in cassava fermentation after lactic acid bacteria (Oyewole and Odunfa, 1990). According to Oyewole (2001) yeasts play an important role in the survival and activity of lactic acid bacteria during fermentation process, as they are involved in cassava starch hydrolysis into simple sugars. *Saccharomyces cerevisiae* is one of the microbes of food safety. It is widely cultured on several agricultural feedstocks. The frequency of *S. cerevisiae* in cassava whey shows it is a promising feedstock for the cultivation of *S. cerevisiae* biomass for energy, enzyme production and other biotechnological applications. Izah (2018b) showed that *S. cerevisiae* can be used to treat cassava mill effluents while generating biomass for other applications. This could help in minimizing the environmental burden associated with cassava waste.

Aspergillus species were also isolated from the cassava whey sampled. *A. niger* is a filamentous ascomycete that is ubiquitous in the environment especially in the soil. It is an important microorganism that produces a wide array of enzymes during the breakdown of lignocelluloses. *A. niger* has been used to convert cassava wastes by semi-solid fermentation into phosphate biofertilizers (Ogbo, 2010). It has also been reported that *A. niger* and *A. flavus* isolated from cassava pulp juice were capable of reducing levels of cyanogenic glucosides in cassava peels to non-toxic levels (Adamafio *et al*., 2010).

Saccharomyces cerevisiae exhibited maximum antagonistic activity among other fungi against pathogenic *E.coli* and this indicates a marked probiotic potential. *S. cerevisiae* is a unicellular organism and one of the most explored in terms of industrial application. Studies have shown that members of *Saccharomyces* genus possess antibacterial and probiotic properties (Fakruddin *et al*., 2017; Fijan, 2014).

Yeasts constitute a large group of organisms that are currently attracting increasing attention from scientists. Their diverse biological activities including antagonistic activity against undesirable bacteria make them promising candidates for a wide range of applications not limited to the food sector (Hatoum *et al*., 2012). In the past two decades, few researches have been conducted to investigate the role of naturally occurring yeasts for inhibiting the growth of food-borne bacteria (Younis *et al*., 2017). Agarry *et al*. (2005) evaluated the antagonistic activity of *Saccharomyces cerevisiae* isolated from cassava products. They showed that *S. cerevisiae* inhibited the growth of *E. coli* and *Staphylococcus aureus*, however the radii of their inhibition zones ranged from 8.00 to 8.90 mm. Antagonisms of microorganism by yeasts have been attributed to pH changes in the medium (as a result of organic acid production), competition of nutrients and secretion of antimicrobial compounds (Hatoum *et al*., 2012).

Conclusion

This study shows that cassava whey has a rich fungal diversity of industrially important species. *Saccharomyces cerevisiae* was indicated as the dominant fungal species in cassava whey and also exhibited the maximum antagonistic activity against pathogenic *E. coli*. Cassava whey can be used for the propagation of yeast biomass.

The discovery of antagonistic property of *S. cerevisiae* will have a significant role in numerous fields such as food and agriculture contributing to product safety by inhibiting food spoilage and pathogenic organisms and also as natural bio-control agents in soil treatments and for preventing pre- and postharvest diseases. The increase in future studies will reveal the ultimate potential of these organisms in different fields of application.

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